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(54) Title: COMPOSITIONS AND METHODS FOR WT1 SPECIFIC IMMUNOTHERAPY

(57) Abstract: Compositions and methods for the therapy of malignant diseases, such as leukemia and cancer, are disclosed. The compositions comprise one or more of a WT1 polynucleotide, a WT1 polypeptide, an antigen-presenting cell presenting a WT1 polypeptide, an antibody that specifically binds to a WT1 polypeptide; or a T cell that specifically reacts with a WT1 polypeptide. Such compositions may be used, for example, for the prevention and treatment of metastatic diseases.



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## COMPOSITIONS AND METHODS FOR WT1 SPECIFIC IMMUNOTHERAPY

## STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

5                    This invention was made in part with government support under NIH SBIR Phase I grant number IR43 CA81752-01A1. The Government may have certain rights in this invention.

## BACKGROUND OF THE INVENTION

Field of the Invention

10                    The present invention relates generally to the immunotherapy of malignant diseases such as leukemia and cancers. The invention is more specifically related to compositions for generating or enhancing an immune response to WT1, and to the use of such compositions for preventing and/or treating malignant diseases.

Description of the Related Art

15                    Cancer and leukemia are significant health problems in the United States and throughout the world. Although advances have been made in detection and treatment of such diseases, no vaccine or other universally successful method for prevention or treatment of cancer and leukemia is currently available. Management of the diseases currently relies on a combination of early diagnosis and aggressive  
20                    treatment, which may include one or more of a variety of treatments such as surgery, radiotherapy, chemotherapy and hormone therapy. The course of treatment for a particular cancer is often selected based on a variety of prognostic parameters, including an analysis of specific tumor markers. However, the use of established markers often leads to a result that is difficult to interpret, and the high mortality continues to be  
25                    observed in many cancer patients.

                      Immunotherapies have the potential to substantially improve cancer and leukemia treatment and survival. Recent data demonstrate that leukemia can be cured by immunotherapy in the context of bone marrow transplantation (*e.g.*, donor lymphocyte infusions). Such therapies may involve the generation or enhancement of

an immune response to a tumor-associated antigen (TAA). However, to date relatively few TAAs are known and the generation of an immune response against such antigens has, with rare exception, not been shown to be therapeutically beneficial.

Accordingly, there is a need in the art for improved methods for  
5 leukemia and cancer prevention and therapy. The present invention fulfills these needs and further provides other related advantages.

#### BRIEF SUMMARY OF THE INVENTION

Briefly stated, this invention provides compositions and methods for the diagnosis and therapy of diseases such as leukemia and cancer. In one aspect, the  
10 present invention provides polypeptides comprising an immunogenic portion of a native WT1, or a variant thereof that differs in one or more substitutions, deletions, additions and/or insertions such that the ability of the variant to react with antigen-specific antisera and/or T-cell lines or clones is not substantially diminished. Within certain  
15 embodiments, the polypeptide comprises no more than 16 consecutive amino acid residues of a native WT1 polypeptide. Within other embodiments, the polypeptide comprises an immunogenic portion of amino acid residues 1 - 174 of a native WT1 polypeptide or a variant thereof, wherein the polypeptide comprises no more than 16 consecutive amino acid residues present within amino acids 175 to 449 of the native  
20 WT1 polypeptide. The immunogenic portion preferably binds to an MHC class I and/or class II molecule. Within certain embodiments, the polypeptide comprises a sequence selected from the group consisting of (a) sequences recited in any one or more of Tables II - XLVI, (b) variants of the foregoing sequences that differ in one or more substitutions, deletions, additions and/or insertions such that the ability of the variant to react with antigen-specific antisera and/or T-cell lines or clones is not substantially  
25 diminished and (c) mimetics of the polypeptides recited above, such that the ability of the mimetic to react with antigen-specific antisera and/or T cell lines or clones is not substantially diminished.

Within other embodiments, the polypeptide comprises a sequence selected from the group consisting of (a) ALLPAVPSL (SEQ ID NO:34),  
30 GATLKGVA A (SEQ ID NO:88), CMTWNQMNL (SEQ ID NOs: 49 and 258),

SCLESQPTI (SEQ ID NOs: 199 and 296), SCLESQPAI (SEQ ID NO:198), NLYQMTSQL (SEQ ID NOs: 147 and 284), ALLPAVSSL (SEQ ID NOs: 35 and 255), RMFPNAPYL (SEQ ID NOs: 185 and 293), VLDFAPPGA (SEQ ID NO:241), VLDFAPPGAS (SEQ ID NO:411), (b) variants of the foregoing sequences that differ in one or more substitutions, deletions, additions and/or insertions such that the ability of the variant to react with antigen-specific antisera and/or T-cell lines or clones is not substantially diminished and (c) mimetics of the polypeptides recited above, such that the ability of the mimetic to react with antigen-specific antisera and/or T cell lines or clones is not substantially diminished. Mimetics may comprises amino acids in combination with one or more amino acid mimetics or may be entirely nonpeptide mimetics.

Within further aspects, the present invention provides polypeptides comprising a variant of an immunogenic portion of a WT1 protein, wherein the variant differs from the immunogenic portion due to substitutions at between 1 and 3 amino acid positions within the immunogenic portion such that the ability of the variant to react with antigen-specific antisera and/or T-cell lines or clones is enhanced relative to a native WT1 protein.

The present invention further provides WT1 polynucleotides that encode a WT1 polypeptide as described above.

Within other aspects, the present invention provides pharmaceutical compositions and vaccines. Pharmaceutical compositions may comprise a polypeptide or mimetic as described above and/or one or more of (i) a WT1 polynucleotide; (ii) an antibody or antigen-binding fragment thereof that specifically binds to a WT1 polypeptide; (iii) a T cell that specifically reacts with a WT1 polypeptide or (iv) an antigen-presenting cell that expresses a WT1 polypeptide, in combination with a pharmaceutically acceptable carrier or excipient. Vaccines comprise a polypeptide as described above and/or one or more of (i) a WT1 polynucleotide, (ii) an antigen-presenting cell that expresses a WT1 polypeptide or (iii) an anti-idiotypic antibody, and a non-specific immune response enhancer. Within certain embodiments, less than 23 consecutive amino acid residues, preferably less than 17 amino acid residues, of a native WT1 polypeptide are present within a WT1 polypeptide employed within such



pharmaceutical compositions and vaccines. The immune response enhancer may be an adjuvant. Preferably, an immune response enhancer enhances a T cell response.

The present invention further provides methods for enhancing or inducing an immune response in a patient, comprising administering to a patient a pharmaceutical composition or vaccine as described above. In certain embodiments, the patient is a human.

The present invention further provides methods for inhibiting the development of a malignant disease in a patient, comprising administering to a patient a pharmaceutical composition or vaccine as described above. Malignant diseases include, but are not limited to leukemias (*e.g.*, acute myeloid, acute lymphocytic and chronic myeloid) and cancers (*e.g.*, breast, lung, thyroid or gastrointestinal cancer or a melanoma). The patient may, but need not, be afflicted with the malignant disease, and the administration of the pharmaceutical composition or vaccine may inhibit the onset of such a disease, or may inhibit progression and/or metastasis of an existing disease.

The present invention further provides, within other aspects, methods for removing cells expressing WT1 from bone marrow and/or peripheral blood or fractions thereof, comprising contacting bone marrow, peripheral blood or a fraction of bone marrow or peripheral blood with T cells that specifically react with a WT1 polypeptide, wherein the step of contacting is performed under conditions and for a time sufficient to permit the removal of WT1 positive cells to less than 10%, preferably less than 5% and more preferably less than 1%, of the number of myeloid or lymphatic cells in the bone marrow, peripheral blood or fraction. Bone marrow, peripheral blood and fractions may be obtained from a patient afflicted with a disease associated with WT1 expression, or may be obtained from a human or non-human mammal not afflicted with such a disease.

Within related aspects, the present invention provides methods for inhibiting the development of a malignant disease in a patient, comprising administering to a patient bone marrow, peripheral blood or a fraction of bone marrow or peripheral blood prepared as described above. Such bone marrow, peripheral blood or fractions may be autologous, or may be derived from a related or unrelated human or non-human animal (*e.g.*, syngeneic or allogeneic).

In other aspects, the present invention provides methods for stimulating (or priming) and/or expanding T cells, comprising contacting T cells with a WT1 polypeptide under conditions and for a time sufficient to permit the stimulation and/or expansion of T cells. Such T cells may be autologous, allogeneic, syngeneic or  
5 unrelated WT1-specific T cells, and may be stimulated *in vitro* or *in vivo*. Expanded T cells may, within certain embodiments, be present within bone marrow, peripheral blood or a fraction of bone marrow or peripheral blood, and may (but need not) be clonal. Within certain embodiments, T cells may be present in a mammal during stimulation and/or expansion. WT1-specific T cells may be used, for example, within  
10 donor lymphocyte infusions.

Within related aspects, methods are provided for inhibiting the development of a malignant disease in a patient, comprising administering to a patient T cells prepared as described above. Such T cells may, within certain embodiments, be autologous, syngeneic or allogeneic.

15 The present invention further provides, within other aspects, methods for monitoring the effectiveness of an immunization or therapy for a malignant disease associated with WT1 expression in a patient. Such methods are based on monitoring antibody, CD4+ T cell and/or CD8+ T cell responses in the patient. Within certain such aspects, a method may comprise the steps of: (a) incubating a first biological sample  
20 with one or more of: (i) a WT1 polypeptide; (ii) a polynucleotide encoding a WT1 polypeptide; or (iii) an antigen presenting cell that expresses a WT1 polypeptide, wherein the first biological sample is obtained from a patient prior to a therapy or immunization, and wherein the incubation is performed under conditions and for a time sufficient to allow immunocomplexes to form; (b) detecting immunocomplexes formed  
25 between the WT1 polypeptide and antibodies in the biological sample that specifically bind to the WT1 polypeptide; (c) repeating steps (a) and (b) using a second biological sample obtained from the same patient following therapy or immunization; and (d) comparing the number of immunocomplexes detected in the first and second biological samples, and therefrom monitoring the effectiveness of the therapy or immunization in  
30 the patient.

Within certain embodiments of the above methods, the step of detecting comprises (a) incubating the immunocomplexes with a detection reagent that is capable of binding to the immunocomplexes, wherein the detection reagent comprises a reporter group, (b) removing unbound detection reagent, and (c) detecting the presence or absence of the reporter group. The detection reagent may comprise, for example, a second antibody, or antigen-binding fragment thereof, capable of binding to the antibodies that specifically bind to the WT1 polypeptide or a molecule such as Protein A. Within other embodiments, a reporter group is bound to the WT1 polypeptide, and the step of detecting comprises removing unbound WT1 polypeptide and subsequently detecting the presence or absence of the reporter group.

Within further aspects, methods for monitoring the effectiveness of an immunization or therapy for a malignant disease associated with WT1 expression in a patient may comprise the steps of: (a) incubating a first biological sample with one or more of: (i) a WT1 polypeptide; (ii) a polynucleotide encoding a WT1 polypeptide; or (iii) an antigen presenting cell that expresses a WT1 polypeptide, wherein the biological sample comprises CD4<sup>+</sup> and/or CD8<sup>+</sup> T cells and is obtained from a patient prior to a therapy or immunization, and wherein the incubation is performed under conditions and for a time sufficient to allow specific activation, proliferation and/or lysis of T cells; (b) detecting an amount of activation, proliferation and/or lysis of the T cells; (c) repeating steps (a) and (b) using a second biological sample comprising CD4<sup>+</sup> and/or CD8<sup>+</sup> T cells, wherein the second biological sample is obtained from the same patient following therapy or immunization; and (d) comparing the amount of activation, proliferation and/or lysis of T cells in the first and second biological samples, and therefrom monitoring the effectiveness of the therapy or immunization in the patient.

The present invention further provides methods for inhibiting the development of a malignant disease associated with WT1 expression in a patient, comprising the steps of: (a) incubating CD4<sup>+</sup> and/or CD8<sup>+</sup> T cells isolated from a patient with one or more of: (i) a WT1 polypeptide; (ii) a polynucleotide encoding a WT1 polypeptide; or (iii) an antigen presenting cell that expresses a WT1 polypeptide, such that the T cells proliferate; and (b) administering to the patient an effective amount of the proliferated T cells, and therefrom inhibiting the development of a malignant

disease in the patient. Within certain embodiments, the step of incubating the T cells may be repeated one or more times.

Within other aspects, the present invention provides methods for inhibiting the development of a malignant disease associated with WT1 expression in a patient, comprising the steps of: (a) incubating CD4<sup>+</sup> and/or CD8<sup>+</sup> T cells isolated from a patient with one or more of: (i) a WT1 polypeptide; (ii) a polynucleotide encoding a WT1 polypeptide; or (iii) an antigen presenting cell that expresses a WT1 polypeptide, such that the T cells proliferate; (b) cloning one or more cells that proliferated; and (c) administering to the patient an effective amount of the cloned T cells.

Within other aspects, methods are provided for determining the presence or absence of a malignant disease associated with WT1 expression in a patient, comprising the steps of: (a) incubating CD4<sup>+</sup> and/or CD8<sup>+</sup> T cells isolated from a patient with one or more of: (i) a WT1 polypeptide; (ii) a polynucleotide encoding a WT1 polypeptide; or (iii) an antigen presenting cell that expresses a WT1 polypeptide; and (b) detecting the presence or absence of specific activation of the T cells, therefrom determining the presence or absence of a malignant disease associated with WT1 expression. Within certain embodiments, the step of detecting comprises detecting the presence or absence of proliferation of the T cells.

Within further aspects, the present invention provides methods for determining the presence or absence of a malignant disease associated with WT1 expression in a patient, comprising the steps of: (a) incubating a biological sample obtained from a patient with one or more of: (i) a WT1 polypeptide; (ii) a polynucleotide encoding a WT1 polypeptide; or (iii) an antigen presenting cell that expresses a WT1 polypeptide, wherein the incubation is performed under conditions and for a time sufficient to allow immunocomplexes to form; and (b) detecting immunocomplexes formed between the WT1 polypeptide and antibodies in the biological sample that specifically bind to the WT1 polypeptide; and therefrom determining the presence or absence of a malignant disease associated with WT1 expression.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

## 5 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts a comparison of the mouse (MO) and human (HU) WT1 protein sequences (SEQ ID NOS: 320 and 319 respectively).

Figure 2 is a Western blot illustrating the detection of WT1 specific antibodies in patients with hematological malignancy (AML). Lane 1 shows molecular weight markers; lane 2 shows a positive control (WT1 positive human leukemia cell line immunoprecipitated with a WT1 specific antibody); lane 3 shows a negative control (WT1 positive cell line immunoprecipitated with mouse sera); and lane 4 shows a WT1 positive cell line immunoprecipitated with sera of a patient with AML. For lanes 2-4, the immunoprecipitate was separated by gel electrophoresis and probed with a WT1 specific antibody.

Figure 3 is a Western blot illustrating the detection of a WT1 specific antibody response in B6 mice immunized with TRAMP-C, a WT1 positive tumor cell line. Lanes 1, 3 and 5 show molecular weight markers, and lanes 2, 4 and 6 show a WT1 specific positive control (N180, Santa Cruz Biotechnology, polypeptide spanning 180 amino acids of the N-terminal region of the WT1 protein, migrating on the Western blot at 52 kD). The primary antibody used was WT180 in lane 2, sera of non-immunized B6 mice in lane 4 and sera of the immunized B6 mice in lane 6.

Figure 4 is a Western blot illustrating the detection of WT1 specific antibodies in mice immunized with representative WT1 peptides. Lanes 1, 3 and 5 show molecular weight markers and lanes 2, 4 and 6 show a WT1 specific positive control (N180, Santa Cruz Biotechnology, polypeptide spanning 180 amino acids of the N-terminal region of the WT1 protein, migrating on the Western blot at 52 kD). The primary antibody used was WT180 in lane 2, sera of non-immunized B6 mice in lane 4 and sera of the immunized B6 mice in lane 6.

Figures 5A to 5C are graphs illustrating the stimulation of proliferative T cell responses in mice immunized with representative WT1 peptides. Thymidine incorporation assays were performed using one T cell line and two different clones, as indicated, and results were expressed as cpm. Controls indicated on the x axis were no antigen (No Ag) and B6/media; antigens used were p6-22 human (p1), p117-139 (p2) or p244-262 human (p3).

Figure 6A and 6B are histograms illustrating the stimulation of proliferative T cell responses in mice immunized with representative WT1 peptides. Three weeks after the third immunization, spleen cells of mice that had been inoculated with Vaccine A or Vaccine B were cultured with medium alone (medium) or spleen cells and medium (B6/no antigen), B6 spleen cells pulsed with the peptides p6-22 (p6), p117-139 (p117), p244-262 (p244) (Vaccine A; Figure 6A) or p287-301 (p287), p299-313 (p299), p421-435 (p421) (Vaccine B; Figure 6B) and spleen cells pulsed with an irrelevant control peptide (irrelevant peptide) at 25ug/ml and were assayed after 96hr for proliferation by ( $^3\text{H}$ ) thymidine incorporation. Bars represent the stimulation index (SI), which is calculated as the mean of the experimental wells divided by the mean of the control (B6 spleen cells with no antigen).

Figures 7A-7D are histograms illustrating the generation of proliferative T-cell lines and clones specific for p117-139 and p6-22. Following *in vivo* immunization, the initial three *in vitro* stimulations (IVS) were carried out using all three peptides of Vaccine A or B, respectively. Subsequent IVS were carried out as single peptide stimulations using only the two relevant peptides p117-139 and p6-22. Clones were derived from both the p6-22 and p117-139 specific T cell lines, as indicated. T cells were cultured with medium alone (medium) or spleen cells and medium (B6/no antigen), B6 spleen cells pulsed with the peptides p6-22 (p6), p117-139 (p117) or an irrelevant control peptide (irrelevant peptide) at 25ug/ml and were assayed after 96hr for proliferation by ( $^3\text{H}$ ) thymidine incorporation. Bars represent the stimulation index (SI), which is calculated as the mean of the experimental wells divided by the mean of the control (B6 spleen cells with no antigen).

Figures 8A and 8B present the results of TSITES Analysis of human WT1 (SEQ ID NO:319) for peptides that have the potential to elicit Th responses.

Regions indicated by "A" are AMPHI midpoints of blocks, "R" indicates residues matching the Rothbard/Taylor motif, "D" indicates residues matching the IAd motif, and 'd' indicates residues matching the IEd motif.

Figures 9A and 9B are graphs illustrating the elicitation of WT1 peptide-specific CTL in mice immunized with WT1 peptides. Figure 9A illustrates the lysis of target cells by allogeneic cell lines and Figure 9B shows the lysis of peptide coated cell lines. In each case, the % lysis (as determined by standard chromium release assays) is shown at three indicated effector:target ratios. Results are provided for lymphoma cells (LSTRA and E10), as well as E10 + p235-243 (E10+P235). E10 cells are also referred to herein as EL-4 cells.

Figures 10A-10D are graphs illustrating the elicitation of WT1 specific CTL, which kill WT1 positive tumor cell lines but do not kill WT1 negative cell lines, following vaccination of B6 mice with WT1 peptide P117. Figure 10A illustrates that T-cells of non-immunized B6 mice do not kill WT1 positive tumor cell lines. Figure 10B illustrates the lysis of the target cells by allogeneic cell lines. Figures 10C and 10D demonstrate the lysis of WT1 positive tumor cell lines, as compared to WT1 negative cell lines in two different experiments. In addition, Figures 10C and 10D show the lysis of peptide-coated cell lines (WT1 negative cell line E10 coated with the relevant WT1 peptide P117). In each case, the % lysis (as determined by standard chromium release assays) is shown at three indicated effector:target ratios. Results are provided for lymphoma cells (E10), prostate cancer cells (TRAMP-C), a transformed fibroblast cell line (BLK-SV40), as well as E10+p117.

Figures 11A and 11B are histograms illustrating the ability of representative peptide P117-139 specific CTL to lyse WT1 positive tumor cells. Three weeks after the third immunization, spleen cells of mice that had been inoculated with the peptides p235-243 or p117-139 were stimulated *in vitro* with the relevant peptide and tested for ability to lyse targets incubated with WT1 peptides as well as WT1 positive and negative tumor cells. The bars represent the mean % specific lysis in chromium release assays performed in triplicate with an E:T ratio of 25:1. Figure 11A shows the cytotoxic activity of the p235-243 specific T cell line against the WT1 negative cell line EL-4 (EL-4, WT1 negative); EL-4 pulsed with the relevant (used for

immunization as well as for restimulation) peptide p235-243 (EL-4+p235); EL-4 pulsed with the irrelevant peptides p117-139 (EL-4+p117), p126-134 (EL-4+p126) or p130-138 (EL-4+p130) and the WT1 positive tumor cells BLK-SV40 (BLK-SV40, WT1 positive) and TRAMP-C (TRAMP-C, WT1 positive), as indicated. Figure 11B shows  
5 cytotoxic activity of the p117-139 specific T cell line against EL-4; EL-4 pulsed with the relevant peptide P117-139 (EL-4+p117) and EL-4 pulsed with the irrelevant peptides p123-131 (EL-4+p123), or p128-136 (EL-4+p128); BLK-SV40 and TRAMP-C, as indicated.

Figures 12A and 12B are histograms illustrating the specificity of lysis of  
10 WT1 positive tumor cells, as demonstrated by cold target inhibition. The bars represent the mean % specific lysis in chromium release assays performed in triplicate with an E:T ratio of 25:1. Figure 12A shows the cytotoxic activity of the p117-139 specific T cell line against the WT1 negative cell line EL-4 (EL-4, WT1 negative); the WT1 positive tumor cell line TRAMP-C (TRAMP-C, WT1 positive); TRAMP-C cells  
15 incubated with a ten-fold excess (compared to the hot target) of EL-4 cells pulsed with the relevant peptide p117-139 (TRAMP-C + p117 cold target) without <sup>51</sup>Cr labeling and TRAMP-C cells incubated with EL-4 pulsed with an irrelevant peptide without <sup>51</sup>Cr labeling (TRAMP-C + irrelevant cold target), as indicated. Figure 12B shows the cytotoxic activity of the p117-139 specific T cell line against the WT1 negative cell line  
20 EL-4 (EL-4, WT1 negative); the WT1 positive tumor cell line BLK-SV40 (BLK-SV40, WT1 positive); BLK-SV40 cells incubated with the relevant cold target (BLK-SV40 + p117 cold target) and BLK-SV40 cells incubated with the irrelevant cold target (BLK-SV40 + irrelevant cold target), as indicated.

Figures 13A-13C are histograms depicting an evaluation of the 9mer  
25 CTL epitope within p117-139. The p117-139 tumor specific CTL line was tested against peptides within aa117-139 containing or lacking an appropriate H-2<sup>b</sup> class I binding motif and following restimulation with p126-134 or p130-138. The bars represent the mean % specific lysis in chromium release assays performed in triplicate with an E:T ratio of 25:1. Figure 13A shows the cytotoxic activity of the p117-139  
30 specific T cell line against the WT1 negative cell line EL-4 (EL-4, WT1 negative) and EL-4 cells pulsed with the peptides p117-139 (EL-4 + p117), p119-127 (EL-4 + p119),



p120-128 (EL-4 + p120), p123-131 (EL-4 + p123), p126-134 (EL-4 + p126), p128-136 (EL-4 + p128), and p130-138 (EL-4 + p130). Figure 13B shows the cytotoxic activity of the CTL line after restimulation with p126-134 against the WT1 negative cell line EL-4, EL-4 cells pulsed with p117-139 (EL-4 + p117), p126-134 (EL-4 + p126) and the  
5 WT1 positive tumor cell line TRAMP-C. Figure 13C shows the cytotoxic activity of the CTL line after restimulation with p130-138 against EL-4, EL-4 cells pulsed with p117-139 (EL-4 + p117), p130-138 (EL-4 + p130) and the WT1 positive tumor cell line TRAMP-C.

Figure 14 depicts serum antibody reactivity to WT1 in 63 patients with  
10 AML. Reactivity of serum antibody to WT1/N-terminus protein was evaluated by ELISA in patients with AML. The first and second lanes represent the positive and negative controls, respectively. The first and second lanes represent the positive and negative controls, respectively. Commercially obtained WT1 specific antibody WT180 was used for the positive control. The next 63 lanes represent results using sera from  
15 each individual patient. The OD values depicted were from ELISA using a 1:500 serum dilution. The figure includes cumulative data from 3 separate experiments.

Figure 15 depicts serum antibody reactivity to WT1 proteins and control proteins in 2 patients with AML. Reactivity of serum antibody to WT1/full-length, WT1N-terminus, TRX and Ra12 proteins was evaluated by ELISA in 2 patients with  
20 AML. The OD values depicted were from ELISA using a 1:500 serum dilution. AML-1 and AML-2 denote serum from 2 of the individual patients in Figure 1 with demonstrated antibody reactivity to WT1/full-length. The WT1 full-length protein was expressed as a fusion protein with Ra12. The WT1/N-terminus protein was expressed as a fusion protein with TRX. The control Ra12 and TRX proteins were purified in a  
25 similar manner. The results confirm that the serum antibody reactivity against the WT1 fusion proteins is directed against the WT1 portions of the protein.

Figure 16 depicts serum antibody reactivity to WT1 in 81 patients with CML. Reactivity of serum antibody to WT1/full-length protein was evaluated by ELISA in patients with AML. The first and second lanes represent the positive and  
30 negative controls, respectively. Commercially obtained WT1 specific antibody WT180 was used for the positive control. The next 81 lanes represent results using sera from

each individual patient. The OD values depicted were from ELISA using a 1:500 serum dilution. The figure includes cumulative data from 3 separate experiments.

Figure 17 depicts serum antibody reactivity to WT1 proteins and control proteins in 2 patients with CML. Reactivity of serum antibody to WT1/full-length, WT1/N-terminus, TRX and Ra12 proteins was evaluated by ELISA in 2 patients with CML. The OD values depicted were from ELISA using a 1:500 serum dilution. CML-1 and CML-2 denote serum from 2 of the individual patients in Figure 3 with demonstrated antibody reactivity to WT1/full-length. The WT1/full-length protein was expressed as a fusion protein with Ra12. The WT1/N-terminus protein was expressed as a fusion protein with TRX. The control Ra12 and TRX proteins were purified in a similar manner. The results confirm that the serum antibody reactivity against the WT1 fusion proteins is directed against the WT1 portions of the protein.

Figure 18 provides the characteristics of the recombinant WT1 proteins used for serological analysis.

Figure 19A-19E is a bar graph depicting the antibody responses in mice elicited by vaccination with different doses of WT1 protein.

Figure 20A and 20B is a bar graph of the proliferative T-cell responses in mice immunized with WT1 protein.

Figure 21 is a photograph of human DC, examined by fluorescent microscopy, expressing WT1 following adeno WT1 and Vaccinia WT1 infection.

Figure 22 is a photograph that demonstrates that WT1 expression in human DC is reproducible following adeno WT1 infection and is not induced by a control Adeno infection.

Figure 23 is a graph of an IFN-gamma ELISPOT assay showing that WT1 whole gene *in vitro* priming elicits WT1 specific T-cell responses.

#### DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is generally directed to compositions and methods for the immunotherapy and diagnosis of malignant diseases. The compositions described herein may include WT1 polypeptides, WT1 polynucleotides, antigen-presenting cells (APC, *e.g.*, dendritic cells) that express a WT1

polypeptide, agents such as antibodies that bind to a WT1 polypeptide and/or immune system cells (*e.g.*, T cells) specific for WT1. WT1 Polypeptides of the present invention generally comprise at least a portion of a Wilms Tumor gene product (WT1) or a variant thereof. Nucleic acid sequences of the subject invention generally comprise  
5 a DNA or RNA sequence that encodes all or a portion of such a polypeptide, or that is complementary to such a sequence. Antibodies are generally immune system proteins, or antigen-binding fragments thereof, that are capable of binding to a portion of a WT1 polypeptide. T cells that may be employed within such compositions are generally T cells (*e.g.*, CD4<sup>+</sup> and/or CD8<sup>+</sup>) that are specific for a WT1 polypeptide. Certain  
10 methods described herein further employ antigen-presenting cells that express a WT1 polypeptide as provided herein.

The present invention is based on the discovery that an immune response raised against a Wilms Tumor (WT) gene product (*e.g.*, WT1) can provide prophylactic and/or therapeutic benefit for patients afflicted with malignant diseases characterized by  
15 increased WT1 gene expression. Such diseases include, but are not limited to, leukemias (*e.g.*, acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL) and childhood ALL), as well as many cancers such as lung, breast, thyroid and gastrointestinal cancers and melanomas. The WT1 gene was originally identified and isolated on the basis of a cytogenetic deletion at  
20 chromosome 11p13 in patients with Wilms' tumor (*see* Call et al., U.S. Patent No. 5,350,840). The gene consists of 10 exons and encodes a zinc finger transcription factor, and sequences of mouse and human WT1 proteins are provided in Figure 1 and SEQ ID NOs: 319 and 320.

#### WT1 Polypeptides

25 Within the context of the present invention, a WT1 polypeptide is a polypeptide that comprises at least an immunogenic portion of a native WT1 (*i.e.*, a WT1 protein expressed by an organism that is not genetically modified), or a variant thereof, as described herein. A WT1 polypeptide may be of any length, provided that it comprises at least an immunogenic portion of a native protein or a variant thereof. In  
30 other words, a WT1 polypeptide may be an oligopeptide (*i.e.*, consisting of a relatively

small number of amino acid residues, such as 8-10 residues, joined by peptide bonds), a full length WT1 protein (*e.g.*, present within a human or non-human animal, such as a mouse) or a polypeptide of intermediate size. Within certain embodiments, the use of WT1 polypeptides that contain a small number of consecutive amino acid residues of a native WT1 polypeptide is preferred. Such polypeptides are preferred for certain uses in which the generation of a T cell response is desired. For example, such a WT1 polypeptide may contain less than 23, preferably no more than 18, and more preferably no more than 15 consecutive amino acid residues, of a native WT1 polypeptide. Polypeptides comprising nine consecutive amino acid residues of a native WT1 polypeptide are generally suitable for such purposes. Additional sequences derived from the native protein and/or heterologous sequences may be present within any WT1 polypeptide, and such sequences may (but need not) possess further immunogenic or antigenic properties. Polypeptides as provided herein may further be associated (covalently or noncovalently) with other polypeptide or non-polypeptide compounds.

An "immunogenic portion," as used herein is a portion of a polypeptide that is recognized (*i.e.*, specifically bound) by a B-cell and/or T-cell surface antigen receptor. Certain preferred immunogenic portions bind to an MHC class I or class II molecule. As used herein, an immunogenic portion is said to "bind to" an MHC class I or class II molecule if such binding is detectable using any assay known in the art. For example, the ability of a polypeptide to bind to MHC class I may be evaluated indirectly by monitoring the ability to promote incorporation of <sup>125</sup>I labeled β2-microglobulin (β2m) into MHC class I/β2m/peptide heterotrimeric complexes (*see* Parker et al., *J. Immunol.* 152:163, 1994). Alternatively, functional peptide competition assays that are known in the art may be employed. Certain immunogenic portions have one or more of the sequences recited within one or more of Tables II - XIV. Representative immunogenic portions include, but are not limited to, RDLNALLPAVPSLGGGG (human WT1 residues 6-22; SEQ ID NO:1), PSQASSGQARMFPNAPYLPSCLE (human and mouse WT1 residues 117-139; SEQ ID NOs: 2 and 3 respectively), GATLKGVAAGSSSVKWTE (human WT1 residues 244-262; SEQ ID NO:4), GATLKGVAA (human WT1 residues 244-252; SEQ ID NO:88), CMTWNQMNL (human and mouse WT1 residues 235-243; SEQ ID NOs: 49 and 258 respectively),

SCLESQPTI (mouse WT1 residues 136-144; SEQ ID NO:296), SCLESQPAI (human WT1 residues 136-144; SEQ ID NO:198), NLYQMTSQL (human and mouse WT1 residues 225-233; SEQ ID NOs: 147 and 284 respectively); ALLPAVSSL (mouse WT1 residues 10-18; SEQ ID NO:255); RMFPNAPYL (human and mouse WT1 residues 126-134; SEQ ID NOs: 185 and 293 respectively), VLDFAPPGA (human WT1 residues 37-45; SEQ ID NO:241), or VLDFAPPGAS (human WT1 residues 37-46; SEQ ID NO:411). Further immunogenic portions are provided herein, and others may generally be identified using well known techniques, such as those summarized in Paul, *Fundamental Immunology*, 3rd ed., 243-247 (Raven Press, 1993) and references cited therein. Representative techniques for identifying immunogenic portions include screening polypeptides for the ability to react with antigen-specific antisera and/or T-cell lines or clones. An immunogenic portion of a native WT1 polypeptide is a portion that reacts with such antisera and/or T-cells at a level that is not substantially less than the reactivity of the full length WT1 (e.g., in an ELISA and/or T-cell reactivity assay). In other words, an immunogenic portion may react within such assays at a level that is similar to or greater than the reactivity of the full length polypeptide. Such screens may generally be performed using methods well known to those of ordinary skill in the art, such as those described in Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988.

Alternatively, immunogenic portions may be identified using computer analysis, such as the Tsites program (see Rothbard and Taylor, *EMBO J.* 7:93-100, 1988; Deavin et al., *Mol. Immunol.* 33:145-155, 1996), which searches for peptide motifs that have the potential to elicit Th responses. CTL peptides with motifs appropriate for binding to murine and human class I or class II MHC may be identified according to BIMAS (Parker et al., *J. Immunol.* 152:163, 1994) and other HLA peptide binding prediction analyses. To confirm immunogenicity, a peptide may be tested using an HLA A2 transgenic mouse model and/or an *in vitro* stimulation assay using dendritic cells, fibroblasts or peripheral blood cells.

As noted above, a composition may comprise a variant of a native WT1 protein. A polypeptide "variant," as used herein, is a polypeptide that differs from a native polypeptide in one or more substitutions, deletions, additions and/or insertions,

such that the immunogenicity of the polypeptide is retained (*i.e.*, the ability of the variant to react with antigen-specific antisera and/or T-cell lines or clones is not substantially diminished relative to the native polypeptide). In other words, the ability of a variant to react with antigen-specific antisera and/or T-cell lines or clones may be enhanced or unchanged, relative to the native polypeptide, or may be diminished by less than 50%, and preferably less than 20%, relative to the native polypeptide. Such variants may generally be identified by modifying one of the above polypeptide sequences and evaluating the reactivity of the modified polypeptide with antisera and/or T-cells as described herein. It has been found, within the context of the present invention, that a relatively small number of substitutions (*e.g.*, 1 to 3) within an immunogenic portion of a WT1 polypeptide may serve to enhance the ability of the polypeptide to elicit an immune response. Suitable substitutions may generally be identified by using computer programs, as described above, and the effect confirmed based on the reactivity of the modified polypeptide with antisera and/or T-cells as described herein. Accordingly, within certain preferred embodiments, a WT1 polypeptide comprises a variant in which 1 to 3 amino acid residues within an immunogenic portion are substituted such that the ability to react with antigen-specific antisera and/or T-cell lines or clones is statistically greater than that for the unmodified polypeptide. Such substitutions are preferably located within an MHC binding site of the polypeptide, which may be identified as described above. Preferred substitutions allow increased binding to MHC class I or class II molecules.

Certain variants contain conservative substitutions. A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydrophobic nature of the polypeptide to be substantially unchanged. Amino acid substitutions may generally be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine;

and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. A variant may also, or alternatively, contain nonconservative changes. Variants  
5 may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the immunogenicity, secondary structure and hydropathic nature of the polypeptide.

In a preferred embodiment, a variant polypeptide of the WT1 N-terminus (amino acids 1-249) is constructed, wherein the variant polypeptide is capable of  
10 binding to an antibody that recognizes full-length WT1 and/or WT1 N-terminus polypeptide. A non-limiting example of an antibody is anti WT1 antibody WT180 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA).

As noted above, WT1 polypeptides may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-  
15 translationally directs transfer of the protein. A polypeptide may also, or alternatively, be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (e.g., poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

WT1 polypeptides may be prepared using any of a variety of well known techniques. Recombinant polypeptides encoded by a WT1 polynucleotide as described herein may be readily prepared from the polynucleotide. In general, any of a variety of expression vectors known to those of ordinary skill in the art may be employed to express recombinant WT1 polypeptides. Expression may be achieved in any  
20 appropriate host cell that has been transformed or transfected with an expression vector containing a DNA molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast and higher eukaryotic cells. Preferably, the host cells employed are *E. coli*, yeast or a mammalian cell line such as COS or CHO. Supernatants from suitable host/vector systems which secrete recombinant protein or  
25 polypeptide into culture media may be first concentrated using a commercially available filter. The concentrate may then be applied to a suitable purification matrix such as an  
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affinity matrix or an ion exchange resin. Finally, one or more reverse phase HPLC steps can be employed to further purify a recombinant polypeptide. Such techniques may be used to prepare native polypeptides or variants thereof. For example, polynucleotides that encode a variant of a native polypeptide may generally be prepared using standard  
5 mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis, and sections of the DNA sequence may be removed to permit preparation of truncated polypeptides.

Certain portions and other variants may also be generated by synthetic means, using techniques well known to those of ordinary skill in the art. For example,  
10 polypeptides having fewer than about 500 amino acids, preferably fewer than about 100 amino acids, and more preferably fewer than about 50 amino acids, may be synthesized. Polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. *See Merrifield, J. Am. Chem. Soc.*  
15 85:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Applied BioSystems, Inc. (Foster City, CA), and may be operated according to the manufacturer's instructions.

In general, polypeptides and polynucleotides as described herein are isolated. An "isolated" polypeptide or polynucleotide is one that is removed from its  
20 original environment. For example, a naturally-occurring protein is isolated if it is separated from some or all of the coexisting materials in the natural system. Preferably, such polypeptides are at least about 90% pure, more preferably at least about 95% pure and most preferably at least about 99% pure. A polynucleotide is considered to be isolated if, for example, it is cloned into a vector that is not a part of the natural  
25 environment.

Within further aspects, the present invention provides mimetics of WT1 polypeptides. Such mimetics may comprise amino acids linked to one or more amino acid mimetics (*i.e.*, one or more amino acids within the WT1 protein may be replaced by an amino acid mimetic) or may be entirely nonpeptide mimetics. An amino acid  
30 mimetic is a compound that is conformationally similar to an amino acid such that it can be substituted for an amino acid within a WT1 polypeptide without substantially



diminishing the ability to react with antigen-specific antisera and/or T cell lines or clones. A nonpeptide mimetic is a compound that does not contain amino acids, and that has an overall conformation that is similar to a WT1 polypeptide such that the ability of the mimetic to react with WT1-specific antisera and/or T cell lines or clones is not substantially diminished relative to the ability of a WT1 polypeptide. Such mimetics may be designed based on standard techniques (*e.g.*, nuclear magnetic resonance and computational techniques) that evaluate the three dimensional structure of a peptide sequence. Mimetics may be designed where one or more of the side chain functionalities of the WT1 polypeptide are replaced by groups that do not necessarily have the same size or volume, but have similar chemical and/or physical properties which produce similar biological responses. It should be understood that, within embodiments described herein, a mimetic may be substituted for a WT1 polypeptide.

Within other illustrative embodiments, a polypeptide may be a fusion polypeptide that comprises multiple polypeptides as described herein, or that comprises at least one polypeptide as described herein and an unrelated sequence, such as a known tumor protein. A fusion partner may, for example, assist in providing T helper epitopes (an immunological fusion partner), preferably T helper epitopes recognized by humans, or may assist in expressing the protein (an expression enhancer) at higher yields than the native recombinant protein. Certain preferred fusion partners are both immunological and expression enhancing fusion partners. Other fusion partners may be selected so as to increase the solubility of the polypeptide or to enable the polypeptide to be targeted to desired intracellular compartments. Still further fusion partners include affinity tags, which facilitate purification of the polypeptide.

Fusion polypeptides may generally be prepared using standard techniques, including chemical conjugation. Preferably, a fusion polypeptide is expressed as a recombinant polypeptide, allowing the production of increased levels, relative to a non-fused polypeptide, in an expression system. Briefly, DNA sequences encoding the polypeptide components may be assembled separately, and ligated into an appropriate expression vector. The 3' end of the DNA sequence encoding one polypeptide component is ligated, with or without a peptide linker, to the 5' end of a DNA sequence encoding the second polypeptide component so that the reading frames

of the sequences are in phase. This permits translation into a single fusion polypeptide that retains the biological activity of both component polypeptides.

A peptide linker sequence may be employed to separate the first and second polypeptide components by a distance sufficient to ensure that each polypeptide folds into its secondary and tertiary structures. Such a peptide linker sequence is incorporated into the fusion polypeptide using standard techniques well known in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea et al., *Gene* 40:39-46, 1985; Murphy et al., *Proc. Natl. Acad. Sci. USA* 83:8258-8262, 1986; U.S. Patent No. 4,935,233 and U.S. Patent No. 4,751,180. The linker sequence may generally be from 1 to about 50 amino acids in length. Linker sequences are not required when the first and second polypeptides have non-essential N-terminal amino acid regions that can be used to separate the functional domains and prevent steric interference.

The ligated DNA sequences are operably linked to suitable transcriptional or translational regulatory elements. The regulatory elements responsible for expression of DNA are located only 5' to the DNA sequence encoding the first polypeptides. Similarly, stop codons required to end translation and transcription termination signals are only present 3' to the DNA sequence encoding the second polypeptide.

The fusion polypeptide can comprise a polypeptide as described herein together with an unrelated immunogenic protein, such as an immunogenic protein capable of eliciting a recall response. Examples of such proteins include tetanus, tuberculosis and hepatitis proteins (*see*, for example, Stoute et al. *New Engl. J. Med.*, 336:86-91, 1997).

In one preferred embodiment, the immunological fusion partner is derived from a *Mycobacterium* sp., such as a *Mycobacterium tuberculosis*-derived Ra12 fragment. Ra12 compositions and methods for their use in enhancing the expression and/or immunogenicity of heterologous polynucleotide/polypeptide sequences is described in U.S. Patent Application 60/158,585, the disclosure of which is incorporated herein by reference in its entirety. Briefly, Ra12 refers to a polynucleotide region that is a subsequence of a *Mycobacterium tuberculosis* MTB32A nucleic acid. MTB32A is a serine protease of 32 KD molecular weight encoded by a gene in virulent and avirulent strains of *M. tuberculosis*. The nucleotide sequence and amino acid sequence of MTB32A have been described (for example, U.S. Patent Application 60/158,585; see also, Skeiky *et al.*, *Infection and Immun.* (1999) 67:3998-4007, incorporated herein by reference). C-terminal fragments of the MTB32A coding sequence express at high levels and remain as soluble polypeptides throughout the purification process. Moreover, Ra12 may enhance the immunogenicity of heterologous immunogenic polypeptides with which it is fused. One preferred Ra12 fusion polypeptide comprises a 14 KD C-terminal fragment corresponding to amino acid residues 192 to 323 of MTB32A. Other preferred Ra12 polynucleotides generally comprise at least about 15 consecutive nucleotides, at least about 30 nucleotides, at least about 60 nucleotides, at least about 100 nucleotides, at least about 200 nucleotides, or at least about 300 nucleotides that encode a portion of a Ra12 polypeptide. Ra12 polynucleotides may comprise a native sequence (*i.e.*, an endogenous sequence that encodes a Ra12 polypeptide or a portion thereof) or may comprise a variant of such a sequence. Ra12 polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions such that the biological activity of the encoded fusion polypeptide is not substantially diminished, relative to a fusion polypeptide comprising a native Ra12 polypeptide. Variants preferably exhibit at least about 70% identity, more preferably at least about 80% identity and most preferably at least about 90% identity to a polynucleotide sequence that encodes a native Ra12 polypeptide or a portion thereof.

Within other preferred embodiments, an immunological fusion partner is derived from protein D, a surface protein of the gram-negative bacterium *Haemophilus*

influenza B (WO 91/18926). Preferably, a protein D derivative comprises approximately the first third of the protein (*e.g.*, the first N-terminal 100-110 amino acids), and a protein D derivative may be lipidated. Within certain preferred embodiments, the first 109 residues of a Lipoprotein D fusion partner is included on the N-terminus to provide the polypeptide with additional exogenous T-cell epitopes and to increase the expression level in *E. coli* (thus functioning as an expression enhancer). The lipid tail ensures optimal presentation of the antigen to antigen presenting cells. Other fusion partners include the non-structural protein from influenzae virus, NS1 (hemagglutinin). Typically, the N-terminal 81 amino acids are used, although different fragments that include T-helper epitopes may be used.

In another embodiment, the immunological fusion partner is the protein known as LYTA, or a portion thereof (preferably a C-terminal portion). LYTA is derived from *Streptococcus pneumoniae*, which synthesizes an N-acetyl-L-alanine amidase known as amidase LYTA (encoded by the *LytA* gene; *Gene* 43:265-292, 1986). LYTA is an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LYTA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the development of *E. coli* C-LYTA expressing plasmids useful for expression of fusion proteins. Purification of hybrid proteins containing the C-LYTA fragment at the amino terminus has been described (*see Biotechnology* 10:795-798, 1992). Within a preferred embodiment, a repeat portion of LYTA may be incorporated into a fusion polypeptide. A repeat portion is found in the C-terminal region starting at residue 178. A particularly preferred repeat portion incorporates residues 188-305.

Yet another illustrative embodiment involves fusion polypeptides, and the polynucleotides encoding them, wherein the fusion partner comprises a targeting signal capable of directing a polypeptide to the endosomal/lysosomal compartment, as described in U.S. Patent No. 5,633,234. An immunogenic polypeptide of the invention, when fused with this targeting signal, will associate more efficiently with MHC class II molecules and thereby provide enhanced in vivo stimulation of CD4<sup>+</sup> T-cells specific for the polypeptide.

The invention provides truncated forms of WT1 polypeptides that can be recombinantly expressed in *E. coli* without the addition of a fusion partner. Examples of these truncated forms are shown in SEQ ID NOs:342-346, and are encoded by polynucleotides shown in SEQ ID NOs:337-341. In variations of these truncations, the first 76 amino acids of WT1 can be fused to the C-terminus of the protein, creating a recombinant protein that is easier to express in *E. coli*. Other hosts in addition to *E. coli* can also be used, such as, for example, *B. megaterium*. The protein can further be prepared without a histidine tag.

In other embodiments, different subunits can be made and fused together in an order which differs from that of native WT1. In addition, fusions can be made with, for example, Ra12. Exemplary fusion proteins are shown in SEQ ID NOs: 332-336 and can be encoded by polynucleotides shown in SEQ ID NOs: 327-331.

#### WT1 Polynucleotides

Any polynucleotide that encodes a WT1 polypeptide as described herein is a WT1 polynucleotide encompassed by the present invention. Such polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present invention, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

WT1 polynucleotides may encode a native WT1 protein, or may encode a variant of WT1 as described herein. Polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions such that the immunogenicity of the encoded polypeptide is not diminished, relative to a native WT1 protein. The effect on the immunogenicity of the encoded polypeptide may generally be assessed as described herein. Preferred variants contain nucleotide substitutions, deletions, insertions and/or additions at no more than 20%, preferably at no more than 10%, of the nucleotide positions that encode an immunogenic portion of a native WT1 sequence. Certain variants are substantially homologous to a native gene, or a portion thereof. Such polynucleotide variants are capable of hybridizing under moderately stringent conditions to a naturally occurring DNA sequence encoding a WT1 polypeptide (or a

complementary sequence). Suitable moderately stringent conditions include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5 X SSC, overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS). Such hybridizing DNA sequences  
5 are also within the scope of this invention.

It will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a WT1 polypeptide. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to  
10 differences in codon usage are specifically contemplated by the present invention.

Once an immunogenic portion of WT1 is identified, as described above, a WT1 polynucleotide may be prepared using any of a variety of techniques. For example, a WT1 polynucleotide may be amplified from cDNA prepared from cells that express WT1. Such polynucleotides may be amplified via polymerase chain reaction  
15 (PCR). For this approach, sequence-specific primers may be designed based on the sequence of the immunogenic portion and may be purchased or synthesized. For example, suitable primers for PCR amplification of a human WT1 gene include: first step - P118: 1434-1414: 5' GAG AGT CAG ACT TGA AAG CAGT 3' (SEQ ID NO:5) and P135: 5' CTG AGC CTC AGC AAA TGG GC 3' (SEQ ID NO:6); second  
20 step - P136: 5' GAG CAT GCA TGG GCT CCG ACG TGC GGG 3' (SEQ ID NO:7) and P137: 5' GGG GTA CCC ACT GAA CGG TCC CCG A 3' (SEQ ID NO:8). Primers for PCR amplification of a mouse WT1 gene include: first step - P138: 5' TCC GAG CCG CAC CTC ATG 3' (SEQ ID NO:9) and P139: 5' GCC TGG GAT GCT GGA CTG 3' (SEQ ID NO:10), second step - P140: 5' GAG CAT GCG ATG GGT  
25 TCC GAC GTG CGG 3' (SEQ ID NO:11) and P141: 5' GGG GTA CCT CAA AGC GCC ACG TGG AGT TT 3' (SEQ ID NO:12).

An amplified portion may then be used to isolate a full length gene from a human genomic DNA library or from a suitable cDNA library, using well known techniques. Alternatively, a full length gene can be constructed from multiple PCR  
30 fragments. WT1 polynucleotides may also be prepared by synthesizing oligonucleotide components, and ligating components together to generate the complete polynucleotide.

WT1 polynucleotides may also be synthesized by any method known in the art, including chemical synthesis (*e.g.*, solid phase phosphoramidite chemical synthesis). Modifications in a polynucleotide sequence may also be introduced using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis (*see* Adelman et al., *DNA* 2:183, 1983). Alternatively, RNA molecules may be generated by *in vitro* or *in vivo* transcription of DNA sequences encoding a WT1 polypeptide, provided that the DNA is incorporated into a vector with a suitable RNA polymerase promoter (such as T7 or SP6). Certain portions may be used to prepare an encoded polypeptide, as described herein. In addition, or alternatively, a portion may be administered to a patient such that the encoded polypeptide is generated *in vivo* (*e.g.*, by transfecting antigen-presenting cells such as dendritic cells with a cDNA construct encoding a WT1 polypeptide, and administering the transfected cells to the patient).

Polynucleotides that encode a WT1 polypeptide may generally be used for production of the polypeptide, *in vitro* or *in vivo*. WT1 polynucleotides that are complementary to a coding sequence (*i.e.*, antisense polynucleotides) may also be used as a probe or to inhibit WT1 expression. cDNA constructs that can be transcribed into antisense RNA may also be introduced into cells of tissues to facilitate the production of antisense RNA.

Any polynucleotide may be further modified to increase stability *in vivo*. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages in the backbone; and/or the inclusion of nontraditional bases such as inosine, queosine and wybutosine, as well as acetyl-, methyl-, thio- and other modified forms of adenine, cytidine, guanine, thymine and uridine.

Nucleotide sequences as described herein may be joined to a variety of other nucleotide sequences using established recombinant DNA techniques. For example, a polynucleotide may be cloned into any of a variety of cloning vectors, including plasmids, phagemids, lambda phage derivatives and cosmids. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors and sequencing vectors. In general, a vector will contain an origin of replication

functional in at least one organism, convenient restriction endonuclease sites and one or more selectable markers. Other elements will depend upon the desired use, and will be apparent to those of ordinary skill in the art.

Within certain embodiments, polynucleotides may be formulated so as to permit entry into a cell of a mammal, and expression therein. Such formulations are particularly useful for therapeutic purposes, as described below. Those of ordinary skill in the art will appreciate that there are many ways to achieve expression of a polynucleotide in a target cell, and any suitable method may be employed. For example, a polynucleotide may be incorporated into a viral vector such as, but not limited to, adenovirus, adeno-associated virus, retrovirus, or vaccinia or other pox virus (*e.g.*, avian pox virus). Techniques for incorporating DNA into such vectors are well known to those of ordinary skill in the art. A retroviral vector may additionally transfer or incorporate a gene for a selectable marker (to aid in the identification or selection of transduced cells) and/or a targeting moiety, such as a gene that encodes a ligand for a receptor on a specific target cell, to render the vector target specific. Targeting may also be accomplished using an antibody, by methods known to those of ordinary skill in the art. cDNA constructs within such a vector may be used, for example, to transfect human or animal cell lines for use in establishing WT1 positive tumor models which may be used to perform tumor protection and adoptive immunotherapy experiments to demonstrate tumor or leukemia-growth inhibition or lysis of such cells.

Other therapeutic formulations for polynucleotides include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*i.e.*, an artificial membrane vesicle). The preparation and use of such systems is well known in the art.

#### Antibodies and Fragments Thereof

The present invention further provides binding agents, such as antibodies and antigen-binding fragments thereof, that specifically bind to a WT1 polypeptide. As used herein, an agent is said to "specifically bind" to a WT1 polypeptide if it reacts at a



detectable level (within, for example, an ELISA) with a WT1 polypeptide, and does not react detectably with unrelated proteins under similar conditions. As used herein, "binding" refers to a noncovalent association between two separate molecules such that a "complex" is formed. The ability to bind may be evaluated by, for example, determining a binding constant for the formation of the complex. The binding constant is the value obtained when the concentration of the complex is divided by the product of the component concentrations. In general, two compounds are said to "bind," in the context of the present invention, when the binding constant for complex formation exceeds about  $10^3$  L/mol. The binding constant maybe determined using methods well known in the art.

Any agent that satisfies the above requirements may be a binding agent. In a preferred embodiment, a binding agent is an antibody or an antigen-binding fragment thereof. Certain antibodies are commercially available from, for example, Santa Cruz Biotechnology (Santa Cruz, CA). Alternatively, antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art. *See, e.g.,* Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In general, antibodies can be produced by cell culture techniques, including the generation of monoclonal antibodies as described herein, or via transfection of antibody genes into suitable bacterial or mammalian cell hosts, in order to allow for the production of recombinant antibodies. In one technique, an immunogen comprising the polypeptide is initially injected into any of a wide variety of mammals (*e.g.*, mice, rats, rabbits, sheep or goats). In this step, the polypeptides of this invention may serve as the immunogen without modification. Alternatively, particularly for relatively short polypeptides, a superior immune response may be elicited if the polypeptide is joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. The immunogen is injected into the animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for the antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol.* 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the  
5 desired specificity (*i.e.*, reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. A variety of fusion techniques may be employed. For example, the spleen cells  
10 and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and their culture  
15 supernatants tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable  
20 vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. The polypeptides of this invention may be used in the purification process in, for example, an affinity chromatography step.

25 Within certain embodiments, the use of antigen-binding fragments of antibodies may be preferred. Such fragments include Fab fragments, which may be prepared using standard techniques. Briefly, immunoglobulins may be purified from rabbit serum by affinity chromatography on Protein A bead columns (Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988) and digested  
30 by papain to yield Fab and Fc fragments. The Fab and Fc fragments may be separated by affinity chromatography on protein A bead columns.

Monoclonal antibodies and fragments thereof may be coupled to one or more therapeutic agents. Suitable agents in this regard include radioactive tracers and chemotherapeutic agents, which may be used, for example, to purge autologous bone marrow *in vitro*). Representative therapeutic agents include radionuclides, differentiation inducers, drugs, toxins, and derivatives thereof. Preferred radionuclides include  $^{90}\text{Y}$ ,  $^{123}\text{I}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{186}\text{Re}$ ,  $^{188}\text{Re}$ ,  $^{211}\text{At}$ , and  $^{212}\text{Bi}$ . Preferred drugs include methotrexate, and pyrimidine and purine analogs. Preferred differentiation inducers include phorbol esters and butyric acid. Preferred toxins include ricin, abrin, diphtheria toxin, cholera toxin, gelonin, *Pseudomonas* exotoxin, *Shigella* toxin, and pokeweed antiviral protein. For diagnostic purposes, coupling of radioactive agents may be used to facilitate tracing of metastases or to determine the location of WT1-positive tumors.

A therapeutic agent may be coupled (*e.g.*, covalently bonded) to a suitable monoclonal antibody either directly or indirectly (*e.g.*, via a linker group). A direct reaction between an agent and an antibody is possible when each possesses a substituent capable of reacting with the other. For example, a nucleophilic group, such as an amino or sulfhydryl group, on one may be capable of reacting with a carbonyl-containing group, such as an anhydride or an acid halide, or with an alkyl group containing a good leaving group (*e.g.*, a halide) on the other.

Alternatively, it may be desirable to couple a therapeutic agent and an antibody via a linker group. A linker group can function as a spacer to distance an antibody from an agent in order to avoid interference with binding capabilities. A linker group can also serve to increase the chemical reactivity of a substituent on an agent or an antibody, and thus increase the coupling efficiency. An increase in chemical reactivity may also facilitate the use of agents, or functional groups on agents, which otherwise would not be possible.

It will be evident to those skilled in the art that a variety of bifunctional or polyfunctional reagents, both homo- and hetero-functional (such as those described in the catalog of the Pierce Chemical Co., Rockford, IL), may be employed as the linker group. Coupling may be effected, for example, through amino groups, carboxyl groups, sulfhydryl groups or oxidized carbohydrate residues. There are numerous references describing such methodology, *e.g.*, U.S. Patent No. 4,671,958, to Rodwell et al.

Where a therapeutic agent is more potent when free from the antibody portion of the immunoconjugates of the present invention, it may be desirable to use a linker group which is cleavable during or upon internalization into a cell. A number of different cleavable linker groups have been described. The mechanisms for the intracellular release of an agent from these linker groups include cleavage by reduction of a disulfide bond (*e.g.*, U.S. Patent No. 4,489,710, to Spitler), by irradiation of a photolabile bond (*e.g.*, U.S. Patent No. 4,625,014, to Senter et al.), by hydrolysis of derivatized amino acid side chains (*e.g.*, U.S. Patent No. 4,638,045, to Kohn et al.), by serum complement-mediated hydrolysis (*e.g.*, U.S. Patent No. 4,671,958, to Rodwell et al.), and acid-catalyzed hydrolysis (*e.g.*, U.S. Patent No. 4,569,789, to Blattler et al.).

It may be desirable to couple more than one agent to an antibody. In one embodiment, multiple molecules of an agent are coupled to one antibody molecule. In another embodiment, more than one type of agent may be coupled to one antibody. Regardless of the particular embodiment, immunoconjugates with more than one agent may be prepared in a variety of ways. For example, more than one agent may be coupled directly to an antibody molecule, or linkers which provide multiple sites for attachment can be used. Alternatively, a carrier can be used. A carrier may bear the agents in a variety of ways, including covalent bonding either directly or via a linker group. Suitable carriers include proteins such as albumins (*e.g.*, U.S. Patent No. 4,507,234, to Kato et al.), peptides and polysaccharides such as aminodextran (*e.g.*, U.S. Patent No. 4,699,784, to Shih et al.). A carrier may also bear an agent by noncovalent bonding or by encapsulation, such as within a liposome vesicle (*e.g.*, U.S. Patent Nos. 4,429,008 and 4,873,088). Carriers specific for radionuclide agents include radiohalogenated small molecules and chelating compounds. For example, U.S. Patent No. 4,735,792 discloses representative radiohalogenated small molecules and their synthesis. A radionuclide chelate may be formed from chelating compounds that include those containing nitrogen and sulfur atoms as the donor atoms for binding the metal, or metal oxide, radionuclide. For example, U.S. Patent No. 4,673,562, to Davison et al. discloses representative chelating compounds and their synthesis.

A variety of routes of administration for the antibodies and immunoconjugates may be used. Typically, administration will be intravenous,

intramuscular, subcutaneous or in the bed of a resected tumor. It will be evident that the precise dose of the antibody/immunoconjugate will vary depending upon the antibody used, the antigen density on the tumor, and the rate of clearance of the antibody.

Also provided herein are anti-idiotypic antibodies that mimic an immunogenic portion of WT1. Such antibodies may be raised against an antibody, or antigen-binding fragment thereof, that specifically binds to an immunogenic portion of WT1, using well known techniques. Anti-idiotypic antibodies that mimic an immunogenic portion of WT1 are those antibodies that bind to an antibody, or antigen-binding fragment thereof, that specifically binds to an immunogenic portion of WT1, as described herein.

#### T Cells

Immunotherapeutic compositions may also, or alternatively, comprise T cells specific for WT1. Such cells may generally be prepared *in vitro* or *ex vivo*, using standard procedures. For example, T cells may be present within (or isolated from) bone marrow, peripheral blood or a fraction of bone marrow or peripheral blood of a mammal, such as a patient, using a commercially available cell separation system, such as the CEPRATE™ system, available from CellPro Inc., Bothell WA (see also U.S. Patent No. 5,240,856; U.S. Patent No. 5,215,926; WO 89/06280; WO 91/16116 and WO 92/07243). Alternatively, T cells may be derived from related or unrelated humans, non-human animals, cell lines or cultures.

T cells may be stimulated with WT1 polypeptide, polynucleotide encoding a WT1 polypeptide and/or an antigen presenting cell (APC) that expresses a WT1 polypeptide. Such stimulation is performed under conditions and for a time sufficient to permit the generation of T cells that are specific for the WT1 polypeptide. Preferably, a WT1 polypeptide or polynucleotide is present within a delivery vehicle, such as a microsphere, to facilitate the generation of antigen-specific T cells. Briefly, T cells, which may be isolated from a patient or a related or unrelated donor by routine techniques (such as by Ficoll/Hypaque density gradient centrifugation of peripheral blood lymphocytes), are incubated with WT1 polypeptide. For example, T cells may be incubated *in vitro* for 2-9 days (typically 4 days) at 37°C with WT1 polypeptide (*e.g.*, 5

to 25 µg/ml) or cells synthesizing a comparable amount of WT1 polypeptide. It may be desirable to incubate a separate aliquot of a T cell sample in the absence of WT1 polypeptide to serve as a control.

T cells are considered to be specific for a WT1 polypeptide if the T cells  
5 kill target cells coated with a WT1 polypeptide or expressing a gene encoding such a polypeptide. T cell specificity may be evaluated using any of a variety of standard techniques. For example, within a chromium release assay or proliferation assay, a stimulation index of more than two fold increase in lysis and/or proliferation, compared to negative controls, indicates T cell specificity. Such assays may be performed, for  
10 example, as described in Chen et al., *Cancer Res.* 54:1065-1070, 1994. Alternatively, detection of the proliferation of T cells may be accomplished by a variety of known techniques. For example, T cell proliferation can be detected by measuring an increased rate of DNA synthesis (e.g., by pulse-labeling cultures of T cells with tritiated thymidine and measuring the amount of tritiated thymidine incorporated into DNA).  
15 Other ways to detect T cell proliferation include measuring increases in interleukin-2 (IL-2) production,  $\text{Ca}^{2+}$  flux, or dye uptake, such as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium. Alternatively, synthesis of lymphokines (such as interferon-gamma) can be measured or the relative number of T cells that can respond to a WT1 polypeptide may be quantified. Contact with a WT1 polypeptide (200 ng/ml - 100  
20 µg/ml, preferably 100 ng/ml - 25 µg/ml) for 3 - 7 days should result in at least a two fold increase in proliferation of the T cells and/or contact as described above for 2-3 hours should result in activation of the T cells, as measured using standard cytokine assays in which a two fold increase in the level of cytokine release (e.g., TNF or IFN- $\gamma$ ) is indicative of T cell activation (see Coligan et al., *Current Protocols in Immunology*,  
25 vol. 1, Wiley Interscience (Greene 1998). WT1 specific T cells may be expanded using standard techniques. Within preferred embodiments, the T cells are derived from a patient or a related or unrelated donor and are administered to the patient following stimulation and expansion.

T cells that have been activated in response to a WT1 polypeptide,  
30 polynucleotide or WT1-expressing APC may be  $\text{CD4}^+$  and/or  $\text{CD8}^+$ . Specific activation of  $\text{CD4}^+$  or  $\text{CD8}^+$  T cells may be detected in a variety of ways. Methods for detecting

specific T cell activation include detecting the proliferation of T cells, the production of cytokines (*e.g.*, lymphokines), or the generation of cytolytic activity (*i.e.*, generation of cytotoxic T cells specific for WT1). For CD4<sup>+</sup> T cells, a preferred method for detecting specific T cell activation is the detection of the proliferation of T cells. For CD8<sup>+</sup> T cells, a preferred method for detecting specific T cell activation is the detection of the generation of cytolytic activity.

For therapeutic purposes, CD4<sup>+</sup> or CD8<sup>+</sup> T cells that proliferate in response to the WT1 polypeptide, polynucleotide or APC can be expanded in number either *in vitro* or *in vivo*. Proliferation of such T cells *in vitro* may be accomplished in a variety of ways. For example, the T cells can be re-exposed to WT1 polypeptide, with or without the addition of T cell growth factors, such as interleukin-2, and/or stimulator cells that synthesize a WT1 polypeptide. The addition of stimulator cells is preferred where generating CD8<sup>+</sup> T cell responses. T cells can be grown to large numbers *in vitro* with retention of specificity in response to intermittent restimulation with WT1 polypeptide. Briefly, for the primary *in vitro* stimulation (IVS), large numbers of lymphocytes (*e.g.*, greater than  $4 \times 10^7$ ) may be placed in flasks with media containing human serum. WT1 polypeptide (*e.g.*, peptide at 10 µg/ml) may be added directly, along with tetanus toxoid (*e.g.*, 5 µg/ml). The flasks may then be incubated (*e.g.*, 37°C for 7 days). For a second IVS, T cells are then harvested and placed in new flasks with 2-3  $\times 10^7$  irradiated peripheral blood mononuclear cells. WT1 polypeptide (*e.g.*, 10 µg/ml) is added directly. The flasks are incubated at 37°C for 7 days. On day 2 and day 4 after the second IVS, 2-5 units of interleukin-2 (IL-2) may be added. For a third IVS, the T cells may be placed in wells and stimulated with the individual's own EBV transformed B cells coated with the peptide. IL-2 may be added on days 2 and 4 of each cycle. As soon as the cells are shown to be specific cytotoxic T cells, they may be expanded using a 10 day stimulation cycle with higher IL-2 (20 units) on days 2, 4 and 6.

Alternatively, one or more T cells that proliferate in the presence of WT1 polypeptide can be expanded in number by cloning. Methods for cloning cells are well known in the art, and include limiting dilution. Responder T cells may be purified from the peripheral blood of sensitized patients by density gradient centrifugation and sheep

red cell rosetting and established in culture by stimulating with the nominal antigen in the presence of irradiated autologous filler cells. In order to generate CD4<sup>+</sup> T cell lines, WT1 polypeptide is used as the antigenic stimulus and autologous peripheral blood lymphocytes (PBL) or lymphoblastoid cell lines (LCL) immortalized by infection with  
5 Epstein Barr virus are used as antigen presenting cells. In order to generate CD8<sup>+</sup> T cell lines, autologous antigen-presenting cells transfected with an expression vector which produces WT1 polypeptide may be used as stimulator cells. Established T cell lines may be cloned 2-4 days following antigen stimulation by plating stimulated T cells at a frequency of 0.5 cells per well in 96-well flat-bottom plates with  $1 \times 10^6$  irradiated PBL  
10 or LCL cells and recombinant interleukin-2 (rIL2) (50 U/ml). Wells with established clonal growth may be identified at approximately 2-3 weeks after initial plating and restimulated with appropriate antigen in the presence of autologous antigen-presenting cells, then subsequently expanded by the addition of low doses of rIL2 (10 U/ml) 2-3 days following antigen stimulation. T cell clones may be maintained in 24-well plates  
15 by periodic restimulation with antigen and rIL2 approximately every two weeks.

Within certain embodiments, allogeneic T-cells may be primed (*i.e.*, sensitized to WT1) *in vivo* and/or *in vitro*. Such priming may be achieved by contacting T cells with a WT1 polypeptide, a polynucleotide encoding such a polypeptide or a cell producing such a polypeptide under conditions and for a time sufficient to permit the  
20 priming of T cells. In general, T cells are considered to be primed if, for example, contact with a WT1 polypeptide results in proliferation and/or activation of the T cells, as measured by standard proliferation, chromium release and/or cytokine release assays as described herein. A stimulation index of more than two fold increase in proliferation or lysis, and more than three fold increase in the level of cytokine, compared to negative  
25 controls, indicates T-cell specificity. Cells primed *in vitro* may be employed, for example, within a bone marrow transplantation or as donor lymphocyte infusion.

T cells specific for WT1 can kill cells that express WT1 protein. Introduction of genes encoding T-cell receptor (TCR) chains for WT1 are used as a means to quantitatively and qualitatively improve responses to WT1 bearing leukemia  
30 and cancer cells. Vaccines to increase the number of T cells that can react to WT1 positive cells are one method of targeting WT1 bearing cells. T cell therapy with T



cells specific for WT1 is another method. An alternative method is to introduce the TCR chains specific for WT1 into T cells or other cells with lytic potential. In a suitable embodiment, the TCR alpha and beta chains are cloned out from a WT1 specific T cell line and used for adoptive T cell therapy, such as described in  
5 WO96/30516, incorporated herein by reference.

#### Pharmaceutical Compositions and Vaccines

Within certain aspects, polypeptides, polynucleotides, antibodies and/or T cells may be incorporated into pharmaceutical compositions or vaccines. Alternatively, a pharmaceutical composition may comprise an antigen-presenting cell  
10 (*e.g.*, a dendritic cell) transfected with a WT1 polynucleotide such that the antigen presenting cell expresses a WT1 polypeptide. Pharmaceutical compositions comprise one or more such compounds or cells and a physiologically acceptable carrier or excipient. Certain vaccines may comprise one or more such compounds or cells and a  
15 non-specific immune response enhancer, such as an adjuvant or a liposome (into which the compound is incorporated). Pharmaceutical compositions and vaccines may additionally contain a delivery system, such as biodegradable microspheres which are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109. Pharmaceutical compositions and vaccines within the scope of the present invention may also contain other compounds, which may be biologically active or inactive.

20 Within certain embodiments, pharmaceutical compositions and vaccines are designed to elicit T cell responses specific for a WT1 polypeptide in a patient, such as a human. In general, T cell responses may be favored through the use of relatively short polypeptides (*e.g.*, comprising less than 23 consecutive amino acid residues of a native WT1 polypeptide, preferably 4-16 consecutive residues, more preferably 8-16  
25 consecutive residues and still more preferably 8-10 consecutive residues. Alternatively, or in addition, a vaccine may comprise a non-specific immune response enhancer that preferentially enhances a T cell response. In other words, the immune response enhancer may enhance the level of a T cell response to a WT1 polypeptide by an amount that is proportionally greater than the amount by which an antibody response is  
30 enhanced. For example, when compared to a standard oil based adjuvant, such as CFA,

an immune response enhancer that preferentially enhances a T cell response may enhance a proliferative T cell response by at least two fold, a lytic response by at least 10%, and/or T cell activation by at least two fold compared to WT1-negative control cell lines, while not detectably enhancing an antibody response. The amount by which a  
5 T cell or antibody response to a WT1 polypeptide is enhanced may generally be determined using any representative technique known in the art, such as the techniques provided herein.

A pharmaceutical composition or vaccine may contain DNA encoding one or more of the polypeptides as described above, such that the polypeptide is  
10 generated *in situ*. As noted above, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacterial and viral expression systems and mammalian expression systems. Appropriate nucleic acid expression systems contain the necessary DNA, cDNA or RNA sequences for expression in the patient (such as a suitable promoter and  
15 terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as *Bacillus-Calmette-Guerrin*) that expresses an immunogenic portion of the polypeptide on its cell surface. In a preferred embodiment, the DNA may be introduced using a viral expression system (*e.g.*, vaccinia or other pox virus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic (defective), replication  
20 competent virus. Techniques for incorporating DNA into such expression systems are well known to those of ordinary skill in the art. The DNA may also be "naked," as described, for example, in Ulmer et al., *Science* 259:1745-1749, 1993 and reviewed by Cohen, *Science* 259:1691-1692, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the  
25 cells.

As noted above, a pharmaceutical composition or vaccine may comprise an antigen-presenting cell that expresses a WT1 polypeptide. For therapeutic purposes, as described herein, the antigen presenting cell is preferably an autologous dendritic cell. Such cells may be prepared and transfected using standard techniques, such as  
30 those described by Reeves et al., *Cancer Res.* 56:5672-5677, 1996; Tuting et al., *J. Immunol.* 160:1139-1147, 1998; and Nair et al., *Nature Biotechnol.* 16:364-369, 1998).

Expression of a WT1 polypeptide on the surface of an antigen-presenting cell may be confirmed by *in vitro* stimulation and standard proliferation as well as chromium release assays, as described herein.

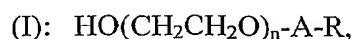
While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. Compositions of the present invention may be formulated for any appropriate manner of administration, including for example, topical, oral, nasal, intravenous, intracranial, intraperitoneal, subcutaneous or intramuscular administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (*e.g.*, polylactate polyglycolate) may also be employed as carriers for the pharmaceutical compositions of this invention. For certain topical applications, formulation as a cream or lotion, using well known components, is preferred.

Such compositions may also comprise buffers (*e.g.*, neutral buffered saline or phosphate buffered saline), carbohydrates (*e.g.*, glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, chelating agents such as EDTA or glutathione, adjuvants (*e.g.*, aluminum hydroxide) and/or preservatives. Alternatively, compositions of the present invention may be formulated as a lyophilizate. Compounds may also be encapsulated within liposomes using well known technology. In one embodiment of the present invention, compositions comprise a buffer comprising one or more sugars including, but not limited to, trehalose, maltose, sucrose, fructose, and glucose, each at a concentration generally between about 1 and 25%, typically between about 7 and 13 %. In a further embodiment, the concentration is between about 8 and about 12%. In yet a further embodiment the concentration is about 10%. In an additional aspect of the present invention, the compositions may comprise ethanolamine; cysteine; or Polysorbate-80, generally at concentrations effective for enhancing the efficacy, stability and/or solubility of the formulation.

Any of a variety of non-specific immune response enhancers, such as adjuvants, may be employed in the vaccines of this invention. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a stimulator of immune responses, such as lipid A, *Bordetella pertussis* or *Mycobacterium tuberculosis* derived proteins. Suitable non-specific immune response enhancers include alum-based adjuvants (*e.g.*, Alhydrogel, Rehydralgel, aluminum phosphate, Algamulin, aluminum hydroxide); oil based adjuvants (Freund's adjuvant (FA), Specol, RIBI, TiterMax, Montanide ISA50 or Montanide ISA 720 (Seppic, France); cytokines (*e.g.*, GM-CSF or Flt3-ligand); microspheres; nonionic block copolymer-based adjuvants; dimethyl dioctadecyl ammoniumbromide (DDA) based adjuvants AS-1, AS-2 (Smith Kline Beecham); Ribi Adjuvant system based adjuvants; QS21 (Aquila); saponin based adjuvants (crude saponin, the saponin Quil A ); muramyl dipeptide (MDP) based adjuvants such as SAF (Syntex adjuvant in its microfluidized form (SAF-m)); dimethyl-dioctadecyl ammonium bromide (DDA); human complement based adjuvants *m. vaccae* and derivatives; immune stimulating complex (iscom) based adjuvants; inactivated toxins; and attenuated infectious agents (such as *M. tuberculosis*).

Additional illustrative adjuvants for use in the pharmaceutical compositions of the invention include, SAF (Chiron, California, United States), ISCOMS (CSL), MF-59 (Chiron), the SBAS series of adjuvants (*e.g.*, SBAS-2 or SBAS-4, available from SmithKline Beecham, Rixensart, Belgium), Detox (Enhanzyn<sup>®</sup>) (Corixa, Hamilton, MT), RC-529 (Corixa, Hamilton, MT) and other aminoalkyl glucosaminide 4-phosphates (AGPs), such as those described in pending U.S. Patent Application Serial Nos. 08/853,826 and 09/074,720, the disclosures of which are incorporated herein by reference in their entireties, and polyoxyethylene ether adjuvants such as those described in WO 99/52549A1.

Other preferred adjuvants include adjuvant molecules of the general formula



wherein, n is 1-50, A is a bond or  $-\text{C}(\text{O})-$ , R is  $\text{C}_{1-50}$  alkyl or Phenyl  $\text{C}_{1-50}$  alkyl.

One embodiment of the present invention consists of a vaccine formulation comprising a polyoxyethylene ether of general formula (I), wherein  $n$  is between 1 and 50, preferably 4-24, most preferably 9; the  $R$  component is  $C_{1-50}$ , preferably  $C_4-C_{20}$  alkyl and most preferably  $C_{12}$  alkyl, and  $A$  is a bond. The concentration of the polyoxyethylene ethers should be in the range 0.1-20%, preferably from 0.1-10%, and most preferably in the range 0.1-1%. Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether, polyoxyethylene-9-stearyl ether, polyoxyethylene-8-stearyl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether. Polyoxyethylene ethers such as polyoxyethylene lauryl ether are described in the Merck index (12<sup>th</sup> edition: entry 7717). These adjuvant molecules are described in WO 99/52549.

The polyoxyethylene ether according to the general formula (I) above may, if desired, be combined with another adjuvant. For example, a preferred adjuvant combination is preferably with CpG as described in the pending UK patent application GB 9820956.2.

As noted above, within certain embodiments, immune response enhancers are chosen for their ability to preferentially elicit or enhance a T cell response (*e.g.*,  $CD4^+$  and/or  $CD8^+$ ) to a WT1 polypeptide. Such immune response enhancers are well known in the art, and include (but are not limited to) Montanide ISA50, Seppic MONTANIDE ISA 720, cytokines (*e.g.*, GM-CSF, Flt3-ligand), microspheres, dimethyl dioctadecyl ammoniumbromide (DDA) based adjuvants, AS-1 (Smith Kline Beecham), AS-2 (Smith Kline Beecham), Ribi Adjuvant system based adjuvants, QS21 (Aquila), saponin based adjuvants (crude saponin, the saponin Quil A), Syntex adjuvant in its microfluidized form (SAF-m), MV, ddMV (Genesis), immune stimulating complex (iscom) based adjuvants and inactivated toxins.

In another aspect of the present invention, compositions may comprise adjuvants for eliciting a predominantly Th1-type response. Certain preferred adjuvants for eliciting a predominantly Th1-type response include, for example, a combination of monophosphoryl lipid A, preferably 3-de-O-acylated monophosphoryl lipid A, together with an aluminum salt. MPL<sup>®</sup> adjuvants, such as MPL-SE, are available from Corixa

Corporation (Seattle, WA; *see*, for example, US Patent Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094, incorporated herein in their entirety). CpG-containing oligonucleotides (in which the CpG dinucleotide is unmethylated) also induce a predominantly Th1 response. Such oligonucleotides are well known and are described, for example, in WO 96/02555, WO 99/33488 and U.S. Patent Nos. 6,008,200 and 5,856,462. Immunostimulatory DNA sequences are also described, for example, by Sato et al., *Science* 273:352, 1996. Another preferred adjuvant comprises a saponin, such as Quil A, or derivatives thereof, including QS21 and QS7 (Aquila Biopharmaceuticals Inc., Framingham, MA); Escin; Digitonin; or *Gypsophila* or *Chenopodium quinoa* saponins. Other preferred formulations include more than one saponin in the adjuvant combinations of the present invention, for example combinations of at least two of the following group comprising QS21, QS7, Quil A,  $\beta$ -escin, or digitonin.

The compositions and vaccines described herein may be administered as part of a sustained release formulation (*i.e.*, a formulation such as a capsule or sponge that effects a slow release of compound following administration). Such formulations may generally be prepared using well known technology and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain a polypeptide, polynucleotide, antibody or cell dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane. Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of active component release. The amount of active compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

#### Therapy of Malignant Diseases

In further aspects of the present invention, the compositions and vaccines described herein may be used to inhibit the development of malignant diseases (*e.g.*, progressive or metastatic diseases or diseases characterized by small tumor burden such

as minimal residual disease). In general, such methods may be used to prevent, delay or treat a disease associated with WT1 expression. In other words, therapeutic methods provided herein may be used to treat an existing WT1-associated disease, or may be used to prevent or delay the onset of such a disease in a patient who is free of disease or  
5 who is afflicted with a disease that is not yet associated with WT1 expression.

As used herein, a disease is "associated with WT1 expression" if diseased cells (*e.g.*, tumor cells) at some time during the course of the disease generate detectably higher levels of a WT1 polypeptide than normal cells of the same tissue. Association of WT1 expression with a malignant disease does not require that WT1 be  
10 present on a tumor. For example, overexpression of WT1 may be involved with initiation of a tumor, but the protein expression may subsequently be lost. Alternatively, a malignant disease that is not characterized by an increase in WT1 expression may, at a later time, progress to a disease that is characterized by increased WT1 expression. Accordingly, any malignant disease in which diseased cells formerly  
15 expressed, currently express or are expected to subsequently express increased levels of WT1 is considered to be "associated with WT1 expression."

Immunotherapy may be performed using any of a variety of techniques, in which compounds or cells provided herein function to remove WT1-expressing cells from a patient. Such removal may take place as a result of enhancing or inducing an  
20 immune response in a patient specific for WT1 or a cell expressing WT1. Alternatively, WT1-expressing cells may be removed *ex vivo* (*e.g.*, by treatment of autologous bone marrow, peripheral blood or a fraction of bone marrow or peripheral blood). Fractions of bone marrow or peripheral blood may be obtained using any standard technique in the art.

25 Within such methods, pharmaceutical compositions and vaccines may be administered to a patient. As used herein, a "patient" refers to any warm-blooded animal, preferably a human. A patient may or may not be afflicted with a malignant disease. Accordingly, the above pharmaceutical compositions and vaccines may be used to prevent the onset of a disease (*i.e.*, prophylactically) or to treat a patient afflicted  
30 with a disease (*e.g.*, to prevent or delay progression and/or metastasis of an existing disease). A patient afflicted with a disease may have a minimal residual disease (*e.g.*, a

low tumor burden in a leukemia patient in complete or partial remission or a cancer patient following reduction of the tumor burden after surgery radiotherapy and/or chemotherapy). Such a patient may be immunized to inhibit a relapse (*i.e.*, prevent or delay the relapse, or decrease the severity of a relapse). Within certain preferred  
5 embodiments, the patient is afflicted with a leukemia (*e.g.*, AML, CML, ALL or childhood ALL), a myelodysplastic syndrome (MDS) or a cancer (*e.g.*, gastrointestinal, lung, thyroid or breast cancer or a melanoma), where the cancer or leukemia is WT1 positive (*i.e.*, reacts detectably with an anti-WT1 antibody, as provided herein or expresses WT1 mRNA at a level detectable by RT-PCR, as described herein) or suffers  
10 from an autoimmune disease directed against WT1-expressing cells.

Other diseases associated with WT1 overexpression include kidney cancer (such as renal cell carcinoma, or Wilms tumor), as described in Satoh F., et al., *Pathol. Int.* 50(6):458-71(2000), and Campbell C. E. et al., *Int. J. Cancer* 78(2):182-8 (1998); and mesothelioma, as described in Amin, K.M. et al., *Am. J. Pathol.* 146(2):344-56 (1995). Harada et al. (*Mol. Urol.* 3(4):357-364 (1999) describe WT1  
15 gene expression in human testicular germ-cell tumors. Nonomura et al. *Hinyokika Kyo* 45(8):593-7 (1999) describe molecular staging of testicular cancer using polymerase chain reaction of the testicular cancer-specific genes. Shimizu et al., *Int. J. Gynecol. Pathol.* 19(2):158-63 (2000) describe the immunohistochemical detection of  
20 the Wilms' tumor gene (WT1) in epithelial ovarian tumors.

WT1 overexpression was also described in desmoplastic small round cell tumors, by Barnoud, R. et al., *Am. J. Surg. Pathol.* 24(6):830-6 (2000); and *Pathol. Res. Pract.* 194(10):693-700 (1998). WT1 overexpression in glioblastoma and other cancer was described by Menssen, H.D. et al., *J. Cancer Res. Clin. Oncol.* 126(4):226-32  
25 (2000), "Wilms' tumor gene (WT1) expression in lung cancer, colon cancer and glioblastoma cell lines compared to freshly isolated tumor specimens." Other diseases showing WT1 overexpression include EBV associated diseases, such as Burkitt's lymphoma and nasopharyngeal cancer (Spinsanti P. et al., *Leuk. Lymphoma* 38(5-6):611-9 (2000), "Wilms' tumor gene expression by normal and malignant human B  
30 lymphocytes."



In *Leukemia* 14(9):1634-4 (2000), Pan et al., describe *in vitro* IL-12 treatment of peripheral blood mononuclear cells from patients with leukemia or myelodysplastic syndromes, and reported an increase in cytotoxicity and reduction in WT1 gene expression. In *Leukemia* 13(6):891-900 (1999), Patmasiriwat et al. reported  
5 WT1 and GATA1 expression in myelodysplastic syndrome and acute leukemia. In *Leukemia* 13(3):393-9 (1999), Tamaki et al. reported that the Wilms' tumor gene WT1 is a good marker for diagnosis of disease progression of myelodysplastic syndromes. Expression of the Wilms' tumor gene WT1 in solid tumors, and its involvement in tumor cell growth, was discussed in relation to gastric cancer, colon cancer, lung cancer,  
10 breast cancer cell lines, germ cell tumor cell line, ovarian cancer, the uterine cancer, thyroid cancer cell line, hepatocellular carcinoma, in Oji et al., *Jpn. J. Cancer Res.* 90(2):194-204 (1999).

The compositions provided herein may be used alone or in combination with conventional therapeutic regimens such as surgery, irradiation, chemotherapy  
15 and/or bone marrow transplantation (autologous, syngeneic, allogeneic or unrelated). As discussed in greater detail below, binding agents and T cells as provided herein may be used for purging of autologous stem cells. Such purging may be beneficial prior to, for example, bone marrow transplantation or transfusion of blood or components thereof. Binding agents, T cells, antigen presenting cells (APC) and compositions  
20 provided herein may further be used for expanding and stimulating (or priming) autologous, allogeneic, syngeneic or unrelated WT1-specific T-cells *in vitro* and/or *in vivo*. Such WT1-specific T cells may be used, for example, within donor lymphocyte infusions.

Routes and frequency of administration, as well as dosage, will vary  
25 from individual to individual, and may be readily established using standard techniques. In general, the pharmaceutical compositions and vaccines may be administered by injection (*e.g.*, intracutaneous, intramuscular, intravenous or subcutaneous), intranasally (*e.g.*, by aspiration) or orally. In some tumors, pharmaceutical compositions or vaccines may be administered locally (by, for example, rectocoloscopy, gastroscopy,  
30 videoendoscopy, angiography or other methods known in the art). Preferably, between 1 and 10 doses may be administered over a 52 week period. Preferably, 6 doses are

administered, at intervals of 1 month, and booster vaccinations may be given periodically thereafter. Alternate protocols may be appropriate for individual patients. A suitable dose is an amount of a compound that, when administered as described above, is capable of promoting an anti-tumor immune response that is at least 10-50%  
5 above the basal (*i.e.*, untreated) level. Such response can be monitored by measuring the anti-tumor antibodies in a patient or by vaccine-dependent generation of cytolytic effector cells capable of killing the patient's tumor cells *in vitro*. Such vaccines should also be capable of causing an immune response that leads to an improved clinical outcome (*e.g.*, more frequent complete or partial remissions, or longer disease-free  
10 and/or overall survival) in vaccinated patients as compared to non-vaccinated patients. In general, for pharmaceutical compositions and vaccines comprising one or more polypeptides, the amount of each polypeptide present in a dose ranges from about 100 µg to 5 mg. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 mL to about 5 mL.

15 In general, an appropriate dosage and treatment regimen provides the active compound(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit. Such a response can be monitored by establishing an improved clinical outcome (*e.g.*, more frequent complete or partial remissions, or longer disease-free and/or overall survival) in treated patients as compared to non-treated patients.  
20 Increases in preexisting immune responses to WT1 generally correlate with an improved clinical outcome. Such immune responses may generally be evaluated using standard proliferation, cytotoxicity or cytokine assays, which may be performed using samples obtained from a patient before and after treatment.

Within further aspects, methods for inhibiting the development of a  
25 malignant disease associated with WT1 expression involve the administration of autologous T cells that have been activated in response to a WT1 polypeptide or WT1-expressing APC, as described above. Such T cells may be CD4<sup>+</sup> and/or CD8<sup>+</sup>, and may be proliferated as described above. The T cells may be administered to the individual in an amount effective to inhibit the development of a malignant disease. Typically, about  
30  $1 \times 10^9$  to  $1 \times 10^{11}$  T cells/M<sup>2</sup> are administered intravenously, intracavitary or in the bed

of a resected tumor. It will be evident to those skilled in the art that the number of cells and the frequency of administration will be dependent upon the response of the patient.

Within certain embodiments, T cells may be stimulated prior to an autologous bone marrow transplantation. Such stimulation may take place *in vivo* or *in vitro*. For *in vitro* stimulation, bone marrow and/or peripheral blood (or a fraction of bone marrow or peripheral blood) obtained from a patient may be contacted with a WT1 polypeptide, a polynucleotide encoding a WT1 polypeptide and/or an APC that expresses a WT1 polypeptide under conditions and for a time sufficient to permit the stimulation of T cells as described above. Bone marrow, peripheral blood stem cells and/or WT1-specific T cells may then be administered to a patient using standard techniques.

Within related embodiments, T cells of a related or unrelated donor may be stimulated prior to a syngeneic or allogeneic (related or unrelated) bone marrow transplantation. Such stimulation may take place *in vivo* or *in vitro*. For *in vitro* stimulation, bone marrow and/or peripheral blood (or a fraction of bone marrow or peripheral blood) obtained from a related or unrelated donor may be contacted with a WT1 polypeptide, WT1 polynucleotide and/or APC that expresses a WT1 polypeptide under conditions and for a time sufficient to permit the stimulation of T cells as described above. Bone marrow, peripheral blood stem cells and/or WT1-specific T cells may then be administered to a patient using standard techniques.

Within other embodiments, WT1-specific T cells as described herein may be used to remove cells expressing WT1 from autologous bone marrow, peripheral blood or a fraction of bone marrow or peripheral blood (*e.g.*, CD34<sup>+</sup> enriched peripheral blood (PB) prior to administration to a patient). Such methods may be performed by contacting bone marrow or PB with such T cells under conditions and for a time sufficient to permit the reduction of WT1 expressing cells to less than 10%, preferably less than 5% and more preferably less than 1%, of the total number of myeloid or lymphatic cells in the bone marrow or peripheral blood. The extent to which such cells have been removed may be readily determined by standard methods such as, for example, qualitative and quantitative PCR analysis, morphology,

immunohistochemistry and FACS analysis. Bone marrow or PB (or a fraction thereof) may then be administered to a patient using standard techniques.

#### Diagnostic Methods

The present invention further provides methods for detecting a malignant disease associated with WT1 expression, and for monitoring the effectiveness of an immunization or therapy for such a disease. Such methods are based on the discovery, within the present invention, that an immune response specific for WT1 protein can be detected in patients afflicted with such diseases, and that methods which enhance such immune responses may provide a preventive or therapeutic benefit.

To determine the presence or absence of a malignant disease associated with WT1 expression, a patient may be tested for the level of T cells specific for WT1. Within certain methods, a biological sample comprising CD4<sup>+</sup> and/or CD8<sup>+</sup> T cells isolated from a patient is incubated with a WT1 polypeptide, a polynucleotide encoding a WT1 polypeptide and/or an APC that expresses a WT1 polypeptide, and the presence or absence of specific activation of the T cells is detected, as described herein. Suitable biological samples include, but are not limited to, isolated T cells. For example, T cells may be isolated from a patient by routine techniques (such as by Ficoll/Hypaque density gradient centrifugation of peripheral blood lymphocytes). T cells may be incubated *in vitro* for 2-9 days (typically 4 days) at 37°C with WT1 polypeptide (*e.g.*, 5 - 25 µg/ml). It may be desirable to incubate another aliquot of a T cell sample in the absence of WT1 polypeptide to serve as a control. For CD4<sup>+</sup> T cells, activation is preferably detected by evaluating proliferation of the T cells. For CD8<sup>+</sup> T cells, activation is preferably detected by evaluating cytolytic activity. A level of proliferation that is at least two fold greater and/or a level of cytolytic activity that is at least 20% greater than in disease-free patients indicates the presence of a malignant disease associated with WT1 expression. Further correlation may be made, using methods well known in the art, between the level of proliferation and/or cytolytic activity and the predicted response to therapy. In particular, patients that display a higher antibody, proliferative and/or lytic response may be expected to show a greater response to therapy.

Within other methods, a biological sample obtained from a patient is tested for the level of antibody specific for WT1. The biological sample is incubated with a WT1 polypeptide, a polynucleotide encoding a WT1 polypeptide and/or an APC that expresses a WT1 polypeptide under conditions and for a time sufficient to allow immunocomplexes to form. Immunocomplexes formed between the WT1 polypeptide and antibodies in the biological sample that specifically bind to the WT1 polypeptide are then detected. A biological sample for use within such methods may be any sample obtained from a patient that would be expected to contain antibodies. Suitable biological samples include blood, sera, ascites, bone marrow, pleural effusion, and cerebrospinal fluid.

The biological sample is incubated with the WT1 polypeptide in a reaction mixture under conditions and for a time sufficient to permit immunocomplexes to form between the polypeptide and antibodies specific for WT1. For example, a biological sample and WT1 polypeptide may be incubated at 4°C for 24-48 hours.

Following the incubation, the reaction mixture is tested for the presence of immunocomplexes. Detection of immunocomplexes formed between the WT1 polypeptide and antibodies present in the biological sample may be accomplished by a variety of known techniques, such as radioimmunoassays (RIA) and enzyme linked immunosorbent assays (ELISA). Suitable assays are well known in the art and are amply described in the scientific and patent literature (*e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988). Assays that may be used include, but are not limited to, the double monoclonal antibody sandwich immunoassay technique of David et al. (U.S. Patent 4,376,110); monoclonal-polyclonal antibody sandwich assays (Wide et al., in Kirkham and Hunter, eds., *Radioimmunoassay Methods*, E. and S. Livingstone, Edinburgh, 1970); the "western blot" method of Gordon et al. (U.S. Patent 4,452,901); immunoprecipitation of labeled ligand (Brown et al., *J. Biol. Chem.* 255:4980-4983, 1980); enzyme-linked immunosorbent assays as described by, for example, Raines and Ross (*J. Biol. Chem.* 257:5154-5160, 1982); immunocytochemical techniques, including the use of fluorochromes (Brooks et al., *Clin. Exp. Immunol.* 39: 477, 1980); and neutralization of activity (Bowen-Pope et al., *Proc. Natl. Acad. Sci. USA* 81:2396-2400, 1984). Other

immunoassays include, but are not limited to, those described in U.S. Patent Nos.: 3,817,827; 3,850,752; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; and 4,098,876.

For detection purposes, WT1 polypeptide may either be labeled or  
5 unlabeled. Unlabeled WT1 polypeptide may be used in agglutination assays or in combination with labeled detection reagents that bind to the immunocomplexes (*e.g.*, anti-immunoglobulin, protein G, protein A or a lectin and secondary antibodies, or antigen-binding fragments thereof, capable of binding to the antibodies that specifically bind to the WT1 polypeptide). If the WT1 polypeptide is labeled, the reporter group  
10 may be any suitable reporter group known in the art, including radioisotopes, fluorescent groups, luminescent groups, enzymes, biotin and dye particles.

Within certain assays, unlabeled WT1 polypeptide is immobilized on a solid support. The solid support may be any material known to those of ordinary skill in the art to which the polypeptide may be attached. For example, the solid support may  
15 be a test well in a microtiter plate or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681. The polypeptide may be immobilized on the solid support using  
20 a variety of techniques known to those of skill in the art, which are amply described in the patent and scientific literature. In the context of the present invention, the term "immobilization" refers to both noncovalent association, such as adsorption, and covalent attachment (which may be a direct linkage between the antigen and functional groups on the support or may be a linkage by way of a cross-linking agent).  
25 Immobilization by adsorption to a well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the WT1 polypeptide, in a suitable buffer, with the solid support for a suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and about 1 day. In general, contacting a well of a plastic microtiter plate (such as  
30 polystyrene or polyvinylchloride) with an amount of polypeptide ranging from about

10 ng to about 10  $\mu$ g, and preferably about 100 ng to about 1  $\mu$ g, is sufficient to immobilize an adequate amount of polypeptide.

Following immobilization, the remaining protein binding sites on the support are typically blocked. Any suitable blocking agent known to those of ordinary skill in the art, such as bovine serum albumin, Tween 20™ (Sigma Chemical Co., St. Louis, MO), heat-inactivated normal goat serum (NGS), or BLOTTO (buffered solution of nonfat dry milk which also contains a preservative, salts, and an antifoaming agent). The support is then incubated with a biological sample suspected of containing specific antibody. The sample can be applied neat, or, more often, it can be diluted, usually in a buffered solution which contains a small amount (0.1%-5.0% by weight) of protein, such as BSA, NGS, or BLOTTO. In general, an appropriate contact time (*i.e.*, incubation time) is a period of time that is sufficient to detect the presence of antibody that specifically binds WT1 within a sample containing such an antibody. Preferably, the contact time is sufficient to achieve a level of binding that is at least about 95% of that achieved at equilibrium between bound and unbound antibody. Those of ordinary skill in the art will recognize that the time necessary to achieve equilibrium may be readily determined by assaying the level of binding that occurs over a period of time. At room temperature, an incubation time of about 30 minutes is generally sufficient.

Unbound sample may then be removed by washing the solid support with an appropriate buffer, such as PBS containing 0.1% Tween 20™. A detection reagent that binds to the immunocomplexes and that comprises a reporter group may then be added. The detection reagent is incubated with the immunocomplex for an amount of time sufficient to detect the bound antibody. An appropriate amount of time may generally be determined by assaying the level of binding that occurs over a period of time. Unbound detection reagent is then removed and bound detection reagent is detected using the reporter group. The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups

(*e.g.*, horseradish peroxidase, beta-galactosidase, alkaline phosphatase and glucose oxidase) may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of the reaction products. Regardless of the specific method employed, a level of bound detection reagent that is  
5 at least two fold greater than background (*i.e.*, the level observed for a biological sample obtained from a disease-free individual) indicates the presence of a malignant disease associated with WT1 expression.

In general, methods for monitoring the effectiveness of an immunization or therapy involve monitoring changes in the level of antibodies or T cells specific for  
10 WT1 in the patient. Methods in which antibody levels are monitored may comprise the steps of: (a) incubating a first biological sample, obtained from a patient prior to a therapy or immunization, with a WT1 polypeptide, wherein the incubation is performed under conditions and for a time sufficient to allow immunocomplexes to form; (b) detecting immunocomplexes formed between the WT1 polypeptide and antibodies in  
15 the biological sample that specifically bind to the WT1 polypeptide; (c) repeating steps (a) and (b) using a second biological sample taken from the patient following therapy or immunization; and (d) comparing the number of immunocomplexes detected in the first and second biological samples. Alternatively, a polynucleotide encoding a WT1 polypeptide, or an APC expressing a WT1 polypeptide may be employed in place of the  
20 WT1 polypeptide. Within such methods, immunocomplexes between the WT1 polypeptide encoded by the polynucleotide, or expressed by the APC, and antibodies in the biological sample are detected.

Methods in which T cell activation and/or the number of WT1 specific precursors are monitored may comprise the steps of: (a) incubating a first biological  
25 sample comprising CD4<sup>+</sup> and/or CD8<sup>+</sup> cells (*e.g.*, bone marrow, peripheral blood or a fraction thereof), obtained from a patient prior to a therapy or immunization, with a WT1 polypeptide, wherein the incubation is performed under conditions and for a time sufficient to allow specific activation, proliferation and/or lysis of T cells; (b) detecting an amount of activation, proliferation and/or lysis of the T cells; (c) repeating steps (a)  
30 and (b) using a second biological sample comprising CD4<sup>+</sup> and/or CD8<sup>+</sup> T cells, and taken from the same patient following therapy or immunization; and (d) comparing the



amount of activation, proliferation and/or lysis of T cells in the first and second biological samples. Alternatively, a polynucleotide encoding a WT1 polypeptide, or an APC expressing a WT1 polypeptide may be employed in place of the WT1 polypeptide.

5 A biological sample for use within such methods may be any sample obtained from a patient that would be expected to contain antibodies, CD4+ T cells and/or CD8+ T cells. Suitable biological samples include blood, sera, ascites, bone marrow, pleural effusion and cerebrospinal fluid. A first biological sample may be obtained prior to initiation of therapy or immunization or part way through a therapy or vaccination regime. The second biological sample should be obtained in a similar  
10 manner, but at a time following additional therapy or immunization. The second biological sample may be obtained at the completion of, or part way through, therapy or immunization, provided that at least a portion of therapy or immunization takes place between the isolation of the first and second biological samples.

Incubation and detection steps for both samples may generally be  
15 performed as described above. A statistically significant increase in the number of immunocomplexes in the second sample relative to the first sample reflects successful therapy or immunization.

The following Examples are offered by way of illustration and not by way of limitation.

## 20 EXAMPLES

### EXAMPLE 1

#### IDENTIFICATION OF AN IMMUNE RESPONSE TO WT1

#### IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

25 This Example illustrates the identification of an existent immune response in patients with a hematological malignancy.

To evaluate the presence of preexisting WT1 specific antibody responses in patients, sera of patients with acute myelogenous leukemia (AML), acute lymphocytic leukemia (ALL), chronic myelogenous leukemia (CML) and severe  
30 aplastic anemia were analyzed using Western blot analysis. Sera were tested for the ability to immunoprecipitate WT1 from the human leukemic cell line K562 (American

Type Culture Collection, Manassas, VA). In each case, immunoprecipitates were separated by gel electrophoresis, transferred to membrane and probed with the anti WT1 antibody WT180 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). This Western blot analysis identified potential WT1 specific antibodies in patients with hematological malignancy. A representative Western blot showing the results for a patient with AML is shown in Figure 2. A 52 kD protein in the immunoprecipitate generated using the patient sera was recognized by the WT1 specific antibody. The 52 kD protein migrated at the same size as the positive control.

Additional studies analyzed the sera of patients with AML and CML for the presence of antibodies to full-length and truncated WT1 proteins. CDNA constructs representing the human WT1/full-length (aa 1-449), the N-terminus (aa 1-249) (WT1/N-terminus) and C-terminus (aa 267-449) (WT1/C-terminus) region were subcloned into modified pET28 vectors. The WT1/full-length and WT1/N-terminus proteins were expressed as Ra12 fusion proteins. Ra12 is the C-terminal fragment of a secreted Mycobacterium tuberculosis protein, denoted as MTB32B. (Skeiky et al., *Infect Immun.* 67;3998, 1999). The Ra12-WT1/full-length fusion region was cloned 3' to a histidine-tag in a histidine-tag modified pET28 vector. The WT1/N-terminus region was subcloned into a modified pET28 vector that has a 5' histidine-tag followed by the thioredoxin (TRX)-WT1/N-terminus fusion region followed by a 3' histidine-tag. The WT1/C-terminus coding region was subcloned into a modified pET28 vector without a fusion partner containing only the 5' and 3' histidine-tag, followed by a Thrombin and EK site.

BL21 pLysS *E. coli* (Stratagene, La Jolla, CA) were transformed with the three WT1 expression constructs, grown overnight and induced with isopropyl- $\beta$ -D-thiogalactoside (IPTG). WT1 proteins were purified as follows: Cells were harvested and lysed by incubation in 10mM Tris, pH 8.0 with Complete Protease Inhibitor Tablets (Boehringer Mannheim Biochemicals, Indianapolis, IN) at 37°C followed by repeated rounds of sonication. Inclusion bodies were washed twice with 10mM Tris, pH 8.0. Proteins were then purified by metal chelate affinity chromatography over nickel-nitrilotriacetic acid resin (QIAGEN Inc., Valencia, CA; Hochuli et al., *Biologically Active Molecules* :217, 1989) followed by chromatography on a Source Q anion

exchange resin (Amersham Pharmacia Biotech, Upsala, Sweden). The identity of the WT1 proteins was confirmed by N-terminal sequencing.

Sera from adult patients with *de nova* AML or CML were studied for the presence of WT1 specific Ab. Recombinant proteins were adsorbed to TC microwell plates (Nunc, Roskilde, Denmark). Plates were washed with PBS/0.5%Tween 20 and blocked with 1% BSA/PBS/0.1%Tween 20. After washing, serum dilutions were added and incubated overnight at 4°C. Plates were washed and Donkey anti-human IgG-HRP secondary antibody was added (Jackson-Immunochem, West Grove, PA) and incubated for 2h at room temperature. Plates were washed, incubated with TMB Peroxidase substrate solution (Kirkegaard and Perry Laboratories, MA), quenched with 1N H<sub>2</sub>SO<sub>4</sub>, and immediately read (Cyto-Fluor 2350; Millipore, Bedford, MA).

For the serological survey, human sera were tested by ELISA over a range of serial dilutions from 1:50 to 1:20,000. A positive reaction was defined as an OD value of a 1:500 diluted serum that exceeded the mean OD value of sera from normal donors (n=96) by three (WT1/full-length, WT1C-terminus) standard deviations. Due to a higher background in normal donors to the WT1/N-terminus protein a positive reaction to WT1/N-terminus was defined as an OD value of 1:500 diluted serum that exceeded the mean OD value of sera from normal donors by four standard deviations. To verify that the patient Ab response was directed against WT1 and not to the Ra12 or TRX fusion part of the protein or possible *E. coli* contaminant proteins, controls included the Ra12 and TRX protein alone purified in a similar manner. Samples that showed reactivity against the Ra12 and/or TRX proteins were excluded from the analysis.

To evaluate for the presence of immunity to WT1, Ab to recombinant full-length and truncated WT1 proteins in the sera of normal individuals and patients with leukemia were determined. Antibody reactivity was analyzed by ELISA reactivity to WT1/full-length protein, WT1/N-terminus protein and WT1/C-terminus protein.

Only 2 of 96 normal donors had serum antibodies reactive with WT1/full-length protein (Figure 18). One of those individuals had antibody to WT1/N-terminus protein and one had antibody to WT1/C-terminus protein. In contrast, 16 of 63 patients (25%) with AML had serum antibodies reactive with WT1/full-length

protein. By marked contrast, only 2 of 63 patients (3%) had reactivity to WT1/C-terminus protein. Fifteen of 81 patients (19%) with CML had serum antibodies reactive with WT1/full-length protein and 12 of 81 patients (15%) had serum antibodies reactive with WT1/N-terminus. Only 3 of 81 patients (3%) had reactivity to WT1/C-terminus protein. (Figures 16 and 17.)

These data demonstrate that Ab responses to WT1 are detectable in some patients with AML and CML. The greater incidence of antibody in leukemia patients provides strong evidence that immunization to the WT1 protein occurred as a result of patients bearing malignancy that expresses or at some time expressed WT1. Without being limited to a specific theory, it is believed that the observed antibody responses to WT1 most probably result from patients becoming immune to WT1 on their own leukemia cells and provide direct evidence that WT1 can be immunogenic despite being a "self" protein.

The presence of antibody to WT1 strongly implies that concurrent helper T cell responses are also present in the same patients. WT1 is an internal protein. Thus, CTL responses are likely to be the most effective in terms of leukemia therapy and the most toxic arm of immunity. Thus, these data provide evidence that therapeutic vaccines directed against WT1 will be able to elicit an immune response to WT1.

The majority of the antibodies detected were reactive with epitopes within the N-terminus while only a small subgroup of patients showed a weak antibody response to the C-terminus. This is consistent with observations in the animal model, where immunization with peptides derived from the N-terminus elicited antibody, helper T cell and CTL responses, whereas none of the peptides tested from the C-terminus elicited antibody or T cell responses (Gaiger et al., *Blood* 96:1334, 2000).

## EXAMPLE 2

### INDUCTION OF ANTIBODIES TO WT1 IN MICE IMMUNIZED WITH CELL LINES EXPRESSING WT1

This Example illustrates the use of cells expressing WT1 to induce a WT1 specific antibody response *in vivo*.

Detection of existent antibodies to WT1 in patients with leukemia strongly implied that it is possible to immunize to WT1 protein to elicit immunity to WT1. To test whether immunity to WT1 can be generated by vaccination, mice were injected with TRAMP-C, a WT1 positive tumor cell line of B6 origin. Briefly, male B6 mice were immunized with  $5 \times 10^6$  TRAMP-C cells subcutaneously and boosted twice with  $5 \times 10^6$  cells at three week intervals. Three weeks after the final immunization, sera were obtained and single cell suspensions of spleens were prepared in RPMI 1640 medium (GIBCO) with 25 $\mu$ M  $\beta$ -2-mercaptoethanol, 200 units of penicillin per ml, 10mM L-glutamine, and 10% fetal bovine serum.

Following immunization to TRAMP-C, a WT1 specific antibody response in the immunized animals was detectable. A representative Western blot is shown in Figure 3. These results show that immunization to WT1 protein can elicit an immune response to WT1 protein.

### EXAMPLE 3

#### INDUCTION OF TH AND ANTIBODY RESPONSES IN MICE IMMUNIZED WITH WT1 PEPTIDES

This Example illustrates the ability of immunization with WT1 peptides to elicit an immune response specific for WT1.

Peptides suitable for eliciting Ab and proliferative T cell responses were identified according to the Tsites program (Rothbard and Taylor, *EMBO J.* 7:93-100, 1988; Deavin et al., *Mol. Immunol.* 33:145-155, 1996), which searches for peptide motifs that have the potential to elicit Th responses. Peptides shown in Table I were synthesized and sequenced.

Table I

WT1 Peptides

Peptide	Sequence	Comments
Mouse: p6-22	RDLNALLPAVSSLGGGG (SEQ ID NO:13)	1 mismatch relative to human WT1 sequence
Human: p6-22	RDLNALLPAVPSLGGGG (SEQ ID NO:1)	
Human/mouse: p117-139	PSQASSGQARMFPNAPYLPSCLE (SEQ ID NOs: 2 and 3)	

Peptide	Sequence	Comments
Mouse: p244-262	GATLKGMAAGSSSSVKWTE (SEQ ID NO:14)	1 mismatch relative to human WT1 sequence
Human: p244-262	GATLKGVAAGSSSSVKWTE (SEQ ID NO:4)	
Human/mouse: p287-301	RIHTHGVFRGIQDVR (SEQ ID NOs: 15 and 16)	
Mouse: p299-313	VRRVSGVAPTLVRS (SEQ ID NO:17)	1 mismatch relative to human WT1 sequence
Human/mouse: p421-435	CQKKFARSDELVRHH (SEQ ID NOs: 19 and 20)	

For immunization, peptides were grouped as follows:

Group A: p6-22 human: 10.9mg in 1ml (10 $\mu$ l = 100 $\mu$ g)  
p117-139 human/mouse: 7.6mg in 1ml (14 $\mu$ l = 100 $\mu$ g)  
p244-262 human: 4.6mg in 1ml (22 $\mu$ l = 100 $\mu$ g)

Group B: p287-301 human/mouse: 7.2mg in 1ml (14 $\mu$ l = 100 $\mu$ g)  
mouse p299-313: 6.6mg in 1ml (15 $\mu$ l = 100 $\mu$ g)  
p421-435 human/mouse: 3.3mg in 1ml (30 $\mu$ l = 100 $\mu$ g)

Control: (FBL peptide 100 $\mu$ g) + CFA/IFA

Control: (CD45 peptide 100 $\mu$ g) + CFA/IFA

Group A contained peptides present within the amino terminus portion of WT1 (exon 1) and Group B contained peptides present within the carboxy terminus, which contains a four zinc finger region with sequence homology to other DNA-binding proteins. Within group B, p287-301 and p299-313 were derived from exon 7, zinc finger I, and p421-435 was derived from exon 10, zinc finger IV.

B6 mice were immunized with a group of WT1 peptides or with a control peptide. Peptides were dissolved in 1ml sterile water for injection, and B6 mice were immunized 3 times at time intervals of three weeks. Adjuvants used were CFA/IFA, GM-CSF, and Montinide. The presence of antibodies specific for WT1 was then determined as described in Examples 1 and 2, and proliferative T cell responses were evaluated using a standard thymidine incorporation assay, in which cells were cultured in the presence of antigen and proliferation was evaluated by measuring incorporated radioactivity (Chen et al., *Cancer Res.* 54:1065-1070, 1994). In particular,

lymphocytes were cultured in 96-well plates at  $2 \times 10^5$  cells per well with  $4 \times 10^5$  irradiated (3000 rads) syngeneic spleen cells and the designated peptide.

Immunization of mice with the group of peptides designated as Group A elicited an antibody response to WT1 (Figure 4). No antibodies were detected following immunization to Vaccine B, which is consistent with a lack of helper T cell response from immunization with Vaccine B. P117-139 elicited proliferative T cell responses (Figures 5A-5C). The stimulation indices (SI) varied between 8 and 72. Other peptides (P6-22 and P299-313) also were shown to elicit proliferative T cell responses. Immunization with P6-22 resulted in a stimulation index (SI) of 2.3 and immunization with P299-313 resulted in a SI of 3.3. Positive controls included ConA stimulated T cells, as well as T cells stimulated with known antigens, such as CD45 and FBL, and allogeneic T cell lines (DeBruijn et al., *Eur. J. Immunol.* 21:2963-2970, 1991).

Figures 6A and 6B show the proliferative response observed for each of the three peptides within vaccine A (Figure 6A) and vaccine B (Figure 6B). Vaccine A elicited proliferative T cell responses to the immunizing peptides p6-22 and p117-139, with stimulation indices (SI) varying between 3 and 8 (bulk lines). No proliferative response to p244-262 was detected (Figure 6A).

Subsequent *in vitro* stimulations were carried out as single peptide stimulations using only p6-22 and p117-139. Stimulation of the Vaccine A specific T cell line with p117-139 resulted in proliferation to p117-139 with no response to p6-22 (Figure 7A). Clones derived from the line were specific for p117-139 (Figure 7B). By contrast, stimulation of the Vaccine A specific T cell line with p6-22 resulted in proliferation to p6-22 with no response to p117-139 (Figure 7C). Clones derived from the line were specific for p6-22 (Figure 7D).

These results show that vaccination with WT1 peptides can elicit antibody responses to WT1 protein and proliferative T cell responses to the immunizing peptides.

## EXAMPLE 4

## INDUCTION OF CTL RESPONSES IN MICE IMMUNIZED WITH WT1 PEPTIDES

This Example illustrates the ability of WT1 peptides to elicit CTL immunity.

5                   Peptides (9-mers) with motifs appropriate for binding to class I MHC were identified using a BIMAS HLA peptide binding prediction analysis (Parker et al., *J. Immunol.* 152:163, 1994). Peptides identified within such analyses are shown in Tables II - XLIV. In each of these tables, the score reflects the theoretical binding affinity (half-time of dissociation) of the peptide to the MHC molecule indicated.

10                   Peptides identified using the Tsites program (Rothbard and Taylor, *EMBO J.* 7:93-100, 1988; Deavin et al., *Mol. Immunol.* 33:145-155, 1996), which searches for peptide motifs that have the potential to elicit Th responses are further shown in Figures 8A and 8B, and Table XLV.

Table II

15                   Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Human HLA A1

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	137	CLESQPAIR (SEQ ID NO:47)	18.000
2	80	GAEPHEEQC (SEQ ID NO:87)	9.000
3	40	FAPPGASAY (SEQ ID NO:74)	5.000
4	354	QCDFKDCER (SEQ ID NO:162)	5.000
5	2	GSDVRDLNA (SEQ ID NO:101)	3.750
6	152	VTFDGTPSY (SEQ ID NO:244)	2.500
7	260	WTEGQSNHS (SEQ ID NO:247)	2.250
8	409	TSEKPFSCR (SEQ ID NO:232)	1.350
9	73	KQEPSWGGA (SEQ ID NO:125)	1.350
10	386	KTCQRKFSR (SEQ ID NO:128)	1.250
11	37	VLDFAAPGA (SEQ ID NO:241)	1.000
12	325	CAYPGCNKR (SEQ ID NO:44)	1.000
13	232	QLECMTNWQ (SEQ ID NO:167)	0.900
14	272	ESDNHTTPI (SEQ ID NO:71)	0.750
15	366	RSDQLKRHQ (SEQ ID NO:193)	0.750
16	222	SSDNLYQMT (SEQ ID NO:217)	0.750



Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
17	427	RSDELVRHH (SEQ ID NO:191)	0.750
18	394	RSDHLKTHT (SEQ ID NO:192)	0.750
19	317	TSEKRPFMC (SEQ ID NO:233)	0.675
20	213	QALLLRTPY (SEQ ID NO:160)	0.500

Table III

Results of BIMAS HLA Peptide Binding Prediction Analysis for

Binding of Human WT1 Peptides to Human HLA A 0201

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	126	RMFPNAPYL (SEQ ID NO:185)	313.968
2	187	SLGEQQYSV (SEQ ID NO:214)	285.163
3	10	ALLPAVPSL (SEQ ID NO:34)	181.794
4	242	NLGATLKG V (SEQ ID NO:146)	159.970
5	225	NLYQMTSQL (SEQ ID NO:147)	68.360
6	292	GVFRGIQDV (SEQ ID NO:103)	51.790
7	191	QQYSVPPPV (SEQ ID NO:171)	22.566
8	280	ILCGAQYRI (SEQ ID NO:116)	17.736
9	235	CMTWNQMNL (SEQ ID NO:49)	15.428
10	441	NMTKLQLAL (SEQ ID NO:149)	15.428
11	7	DLNALLPAV (SEQ ID NO:58)	11.998
12	227	YQMTSQLEC (SEQ ID NO:251)	8.573
13	239	NQMNLGATL (SEQ ID NO:151)	8.014
14	309	TLVRSASET (SEQ ID NO:226)	7.452
15	408	KTSEKPFSC (SEQ ID NO:129)	5.743
16	340	LQMHSRKHT (SEQ ID NO:139)	4.752
17	228	QMTSQLECM (SEQ ID NO:169)	4.044
18	93	TVHFSGQFT (SEQ ID NO:235)	3.586
19	37	VLDFAPPGA (SEQ ID NO:241)	3.378
20	86	EQCLSAFTV (SEQ ID NO:69)	3.068

Table IV

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Human HLA A 0205

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	10	ALLPAVPSL (SEQ ID NO:34)	42.000
2	292	GVFRGIQDV (SEQ ID NO:103)	24.000
3	126	RMFPNAPYL (SEQ ID NO:185)	21.000
4	225	NLYQMTSQL (SEQ ID NO:147)	21.000
5	239	NQMNLGATL (SEQ ID NO:151)	16.800
6	302	RVPGVAPTL (SEQ ID NO:195)	14.000
7	441	NMTKLQLAL (SEQ ID NO:149)	7.000
8	235	CMTWNQMNL (SEQ ID NO:49)	7.000
9	187	SLGEQQYSV (SEQ ID NO:214)	6.000
10	191	QQYSVPPPV (SEQ ID NO:171)	4.800
11	340	LQMHSRKHT (SEQ ID NO:139)	4.080
12	242	NLGATLKGV (SEQ ID NO:146)	4.000
13	227	YQMTSQLEC (SEQ ID NO:251)	3.600
14	194	SVPPPVYGC (SEQ ID NO:218)	2.000
15	93	TVHFSGQFT (SEQ ID NO:235)	2.000
16	280	ILCGAQYRI (SEQ ID NO:116)	1.700
17	98	GQFTGTAGA (SEQ ID NO:99)	1.200
18	309	TLVRSASET (SEQ ID NO:226)	1.000
19	81	AEPHEEQCL (SEQ ID NO:30)	0.980
20	73	KQEPSWGGA (SEQ ID NO:125)	0.960

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Table V

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Human HLA A24

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	302	RVPGVAPTL (SEQ ID NO:195)	16.800
2	218	RTPYSSDNL (SEQ ID NO:194)	12.000
3	356	DFKDCERRF (SEQ ID NO:55)	12.000
4	126	RMFPNAPYL (SEQ ID NO:185)	9.600
5	326	AYPGCNKRY (SEQ ID NO:42)	7.500

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
6	270	GYESDNHT (SEQ ID NO:106)T	7.500
7	239	NQMNLGATL (SEQ ID NO:151)	7.200
8	10	ALLPAVPSL (SEQ ID NO:34)	7.200
9	130	NAPYLPSC (SEQ ID NO:144)	7.200
10	329	GCNKRYFKL (SEQ ID NO:90)	6.600
11	417	RWPSCQKKF (SEQ ID NO:196)	6.600
12	47	AYGSLGGPA (SEQ ID NO:41)	6.000
13	180	DPMGQQGSL (SEQ ID NO:59)	6.000
14	4	DVRDLNALL (SEQ ID NO:62)	5.760
15	285	QYRIHTHGV (SEQ ID NO:175)	5.000
16	192	QYSVPPPVY (SEQ ID NO:176)	5.000
17	207	DSCTGSQAL (SEQ ID NO:61)	4.800
18	441	NMTKLQLAL (SEQ ID NO:149)	4.800
19	225	NLYQMTSQL (SEQ ID NO:147)	4.000
20	235	CMTWNQMNL (SEQ ID NO:49)	4.000

Table VI

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Human HLA A3

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	436	NMHQRNMTK (SEQ ID NO:148)	40.000
2	240	QMNLGATLK (SEQ ID NO:168)	20.000
3	88	CLSAFTVHF (SEQ ID NO:48)	6.000
4	126	RMFPNAPYL (SEQ ID NO:185)	4.500
5	169	AQFPNHSFK (SEQ ID NO:36)	4.500
6	10	ALLPAVPSL (SEQ ID NO:34)	4.050
7	137	CLESQPAIR (SEQ ID NO:47)	4.000
8	225	NLYQMTSQL (SEQ ID NO:147)	3.000
9	32	AQWAPVLDF (SEQ ID NO:37)	2.700
10	280	ILCGAQYRI (SEQ ID NO:116)	2.700
11	386	KTCQRKFSR (SEQ ID NO:128)	1.800
12	235	CMTWNQMNL (SEQ ID NO:49)	1.200

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
13	441	NMTKLQLAL (SEQ ID NO:149)	1.200
14	152	VTFDGTPSY (SEQ ID NO:244)	1.000
15	187	SLGEQQYSV (SEQ ID NO:214)	0.900
16	383	FQCKTCQRK (SEQ ID NO:80)	0.600
17	292	GVFRGIQDV (SEQ ID NO:103)	0.450
18	194	SVPPPVGVC (SEQ ID NO:218)	0.405
19	287	RIHTHGVFR (SEQ ID NO:182)	0.400
20	263	GQSNHSTGY (SEQ ID NO:100)	0.360

Table VII

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Human HLA A68.1

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	100	FTGTAGACR (SEQ ID NO:84)	100.000
2	386	KTCQRKFSR (SEQ ID NO:128)	50.000
3	368	DQLKRHQRR (SEQ ID NO:60)	30.000
4	312	RSASETSEK (SEQ ID NO:190)	18.000
5	337	LSHLQMHSR (SEQ ID NO:141)	15.000
6	364	FSRSDQLKR (SEQ ID NO:83)	15.000
7	409	TSEKPFSCR (SEQ ID NO:232)	15.000
8	299	DVRRVPGVA (SEQ ID NO:63)	12.000
9	4	DVRDLNALL (SEQ ID NO:62)	12.000
10	118	SQASSGQAR (SEQ ID NO:216)	10.000
11	343	HSRKHTGEK (SEQ ID NO:111)	9.000
12	169	AQFPNHSFK (SEQ ID NO:36)	9.000
13	292	GVFRGIQDV (SEQ ID NO:103)	8.000
14	325	CAYPGCNKR (SEQ ID NO:44)	7.500
15	425	FARSDELVR (SEQ ID NO:75)	7.500
16	354	QCDFKDCER (SEQ ID NO:162)	7.500
17	324	MCAYPGCNK (SEQ ID NO:142)	6.000
18	251	AAGSSSSVK (SEQ ID NO:28)	6.000
19	379	GVKPFQCKT (SEQ ID NO:104)	6.000
20	137	CLESQPAIR (SEQ ID NO:47)	5.000

Table VIIIResults of BIMAS HLA Peptide Binding Prediction Analysis forBinding of Human WT1 Peptides to Human HLA A 1101

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	386	KTCQRKFSR (SEQ ID NO:128)	1.800
2	169	AQFPNHSFK (SEQ ID NO:36)	1.200
3	436	NMHQRNMTK (SEQ ID NO:148)	0.800
4	391	KFSRSDHLK (SEQ ID NO:120)	0.600
5	373	HQRRHTGVK (SEQ ID NO:109)	0.600
6	383	FQCKTCQRK (SEQ ID NO:80)	0.600
7	363	RFSRSDQLK (SEQ ID NO:178)	0.600
8	240	QMNLGATLK (SEQ ID NO:168)	0.400
9	287	RIHTHGVFR (SEQ ID NO:182)	0.240
10	100	FTGTAGACR (SEQ ID NO:84)	0.200
11	324	MCAYPGCNK (SEQ ID NO:142)	0.200
12	251	AAGSSSSVK (SEQ ID NO:28)	0.200
13	415	SCRWPSCQK (SEQ ID NO:201)	0.200
14	118	SQASSGQAR (SEQ ID NO:216)	0.120
15	292	GVFRGIQDV (SEQ ID NO:103)	0.120
16	137	CLESQPAIR (SEQ ID NO:47)	0.080
17	425	FARSDDELVR (SEQ ID NO:75)	0.080
18	325	CAYPGCNKR (SEQ ID NO:44)	0.080
19	312	RSASETSEK (SEQ ID NO:190)	0.060
20	65	PPPPHSFI (SEQ ID NO:156)K	0.060

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Table IXResults of BIMAS HLA Peptide Binding Prediction Analysis forBinding of Human WT1 Peptides to Human HLA A 3101

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	386	KTCQRKFSR (SEQ ID NO:128)	9.000
2	287	RIHTHGVFR (SEQ ID NO:182)	6.000
3	137	CLESQPAIR (SEQ ID NO:47)	2.000
4	118	SQASSGQAR (SEQ ID NO:216)	2.000
5	368	DQLKRHQRR (SEQ ID NO:60)	1.200

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
6	100	FTGTAGACR (SEQ ID NO:84)	1.000
7	293	VFRGIQDVR (SEQ ID NO:238)	0.600
8	325	CAYPGCNKR (SEQ ID NO:44)	0.600
9	169	AQFPNHSFK (SEQ ID NO:36)	0.600
10	279	PILCGAQYR (SEQ ID NO:155)	0.400
11	436	NMHQRNMTK (SEQ ID NO:148)	0.400
12	425	FARSDDELVR (SEQ ID NO:75)	0.400
13	32	AQWAPVLDF (SEQ ID NO:37)	0.240
14	240	QMNLGATLK (SEQ ID NO:168)	0.200
15	354	QCDFKDCER (SEQ ID NO:162)	0.200
16	373	HQRRHTGVK (SEQ ID NO:109)	0.200
17	383	FQCKTCQRK (SEQ ID NO:80)	0.200
18	313	SASETSEKR (SEQ ID NO:197)	0.200
19	358	KDCERRFSR (SEQ ID NO:118)	0.180
20	391	KFSRSDHLK (SEQ ID NO:120)	0.180

Table X

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Human HLA A 3302

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	337	LSHLQMHSR (SEQ ID NO:141)	15.000
2	409	TSEKPFSCR (SEQ ID NO:232)	15.000
3	364	FSRSDQLKR (SEQ ID NO:83)	15.000
4	137	CLESQPAIR (SEQ ID NO:47)	9.000
5	368	DQLKRHQRR (SEQ ID NO:60)	9.000
6	287	RIHTHGVFR (SEQ ID NO:182)	4.500
7	210	TGSQALLR (SEQ ID NO:223)	3.000
8	425	FARSDDELVR (SEQ ID NO:75)	3.000
9	313	SASETSEKR (SEQ ID NO:197)	3.000
10	293	VFRGIQDVR (SEQ ID NO:238)	3.000
11	354	QCDFKDCER (SEQ ID NO:162)	3.000
12	100	FTGTAGACR (SEQ ID NO:84)	3.000
13	118	SQASSGQAR (SEQ ID NO:216)	3.000
14	325	CAYPGCNKR (SEQ ID NO:44)	3.000
15	207	DSCTGSQAL (SEQ ID NO:61)	1.500

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
16	139	ESQPAIRNQ (SEQ ID NO:72)	1.500
17	299	DVRRVPGVA (SEQ ID NO:63)	1.500
18	419	PSCQKKFAR (SEQ ID NO:159)	1.500
19	272	ESDNHTTPI (SEQ ID NO:71)	1.500
20	4	DVRDLNALL (SEQ ID NO:62)	1.500

Table XI

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Human HLA B14

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	362	RRFSRSDQL (SEQ ID NO:187)	1000.000
2	332	KRYFKLSHL (SEQ ID NO:127)	300.000
3	423	KKFARSDEL (SEQ ID NO:122)	150.000
4	390	RKFSRSDHL (SEQ ID NO:183)	150.000
5	439	QRNMTKLQL (SEQ ID NO:173)	20.000
6	329	GCNKRYFKL (SEQ ID NO:90)	10.000
7	10	ALLPAVPSL (SEQ ID NO:34)	10.000
8	180	DPMGQQGSL (SEQ ID NO:59)	9.000
9	301	RRVPGVAPT (SEQ ID NO:189)	6.000
10	126	RMFPNAPYL (SEQ ID NO:185)	5.000
11	371	KRHQRRHTG (SEQ ID NO:126)	5.000
12	225	NLYQMTSQL (SEQ ID NO:147)	5.000
13	144	IRNQGYSTV (SEQ ID NO:117)	4.000
14	429	DELVRHHNM (SEQ ID NO:53)	3.000
15	437	MHQRNMTKL (SEQ ID NO:143)	3.000
16	125	ARMFPNAPY (SEQ ID NO:38)	3.000
17	239	NQMNLGATL (SEQ ID NO:151)	3.000
18	286	YRIHTHGVF (SEQ ID NO:252)	3.000
19	174	HSFKHEDPM (SEQ ID NO:110)	3.000
20	372	RHQRRHTGV (SEQ ID NO:181)	3.000

Table XII

Results of BIMAS HLA Peptide Binding Prediction Analysis for

Binding of Human WT1 Peptides to Human HLA B40

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	81	AEPHEEQCL (SEQ ID NO:30)	40.000
2	429	DELVRHHNM (SEQ ID NO:53)	24.000
3	410	SEKPFSCRW (SEQ ID NO:207)	20.000
4	318	SEKRPFMCA (SEQ ID NO:208)	15.000
5	233	LECMTWNQM (SEQ ID NO:131)	12.000
6	3	SDVRDLNAL (SEQ ID NO:206)	10.000
7	349	GEKPYQCDF (SEQ ID NO:91)	8.000
8	6	RDLNALLPA (SEQ ID NO:177)	5.000
9	85	EEQCLSAFT (SEQ ID NO:65)	4.000
10	315	SETSEKRPF (SEQ ID NO:209)	4.000
11	261	TEGQSNHST (SEQ ID NO:221)	4.000
12	23	GCALPVSGA (SEQ ID NO:89)	3.000
13	38	LDFAPPGAS (SEQ ID NO:130)	3.000
14	273	SDNHTTPIL (SEQ ID NO:204)	2.500
15	206	TDCTGSQA (SEQ ID NO:220)	2.500
16	24	CALPVSGAA (SEQ ID NO:43)	2.000
17	98	GQFTGTAGA (SEQ ID NO:99)	2.000
18	30	GAAQWAPVL (SEQ ID NO:86)	2.000
19	84	HEEQCLSAF (SEQ ID NO:107)	2.000
20	26	LPVSGAAQW (SEQ ID NO:138)	2.000

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Table XIII

Results of BIMAS HLA Peptide Binding Prediction Analysis for

Binding of Human WT1 Peptides to Human HLA B60

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	81	AEPHEEQCL (SEQ ID NO:30)	160.000
2	3	SDVRDLNAL (SEQ ID NO:206)	40.000
3	429	DELVRHHNM (SEQ ID NO:53)	40.000
4	233	LECMTWNQM (SEQ ID NO:131)	22.000
5	273	SDNHTTPIL (SEQ ID NO:204)	20.000



Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
6	209	CTGSQALLL (SEQ ID NO:52)	8.000
7	30	GAAQWAPVL (SEQ ID NO:86)	8.000
8	318	SEKRPFMCA (SEQ ID NO:208)	8.000
9	180	DPMGQQGSL (SEQ ID NO:59)	8.000
10	138	LESQPAIRN (SEQ ID NO:132)	5.280
11	239	NQMNLGATL (SEQ ID NO:151)	4.400
12	329	GCKNRYFKL (SEQ ID NO:90)	4.400
13	130	NAPYLPSCl (SEQ ID NO:144)	4.400
14	85	EEQCLSAFT (SEQ ID NO:65)	4.400
15	208	SCTGSQALL (SEQ ID NO:202)	4.000
16	207	DSCTGSQAL (SEQ ID NO:61)	4.000
17	218	RTPYSSDNL (SEQ ID NO:194)	4.000
18	261	TEGQSNHST (SEQ ID NO:221)	4.000
19	18	LGGGGGCAL (SEQ ID NO:134)	4.000
20	221	YSSDNL YQM (SEQ ID NO:253)	2.200

Table XIV

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Human HLA B61

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	318	SEKRPFMCA (SEQ ID NO:208)	20.000
2	429	DELVRHHNM (SEQ ID NO:53)	16.000
3	298	QDVRRVPGV (SEQ ID NO:164)	10.000
4	81	AEPHEEQCL (SEQ ID NO:30)	8.000
5	233	LECMTWNQM (SEQ ID NO:131)	8.000
6	6	RDLNALLPA (SEQ ID NO:177)	5.500
7	85	EEQCLSAFT (SEQ ID NO:65)	4.000
8	261	TEGQSNHST (SEQ ID NO:221)	4.000
9	206	TDCTGSQA (SEQ ID NO:220)	2.500
10	295	RGIQDVRRV (SEQ ID NO:179)	2.200
11	3	SDVRDLNAL (SEQ ID NO:206)	2.000
12	250	VAAGSSSSV (SEQ ID NO:236)	2.000
13	29	SGAAQWAPV (SEQ ID NO:211)	2.000
14	315	SETSEKRPF (SEQ ID NO:209)	1.600
15	138	LESQPAIRN (SEQ ID NO:132)	1.200

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
16	244	GATLKGVAA (SEQ ID NO:88)	1.100
17	20	GGGGCALPV (SEQ ID NO:92)	1.100
18	440	RNMTKLQLA (SEQ ID NO:186)	1.100
19	23	GCALPVSGA (SEQ ID NO:89)	1.100
20	191	QQYSVPPP (SEQ ID NO:171)	1.000

Table XV

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Human HLA B62

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	146	NQGYSTVTF (SEQ ID NO:150)	211.200
2	32	AQWAPVLDF (SEQ ID NO:37)	96.000
3	263	GQSNHSTGY (SEQ ID NO:100)	96.000
4	88	CLSAFTVHF (SEQ ID NO:48)	96.000
5	17	SLGGGGGCA (SEQ ID NO:215)	9.600
6	239	NQMNLGATL (SEQ ID NO:151)	8.800
7	191	QQYSVPPP (SEQ ID NO:171)	8.000
8	98	GQFTGTAGA (SEQ ID NO:99)	8.000
9	384	QCKTCQRKF (SEQ ID NO:163)	6.000
10	40	FAPPGASAY (SEQ ID NO:74)	4.800
11	227	YQMTSQLEC (SEQ ID NO:251)	4.800
12	187	SLGEQQYSV (SEQ ID NO:214)	4.400
13	86	EQCLSAFTV (SEQ ID NO:69)	4.400
14	152	VTFDGTPSY (SEQ ID NO:244)	4.400
15	101	TGTAGACRY (SEQ ID NO:224)	4.000
16	242	NLGATLKG (SEQ ID NO:146)	4.000
17	92	FTVHFSGQF (SEQ ID NO:85)	4.000
18	7	DLNALLPAV (SEQ ID NO:58)	4.000
19	123	GQARMFPNA (SEQ ID NO:98)	4.000
20	280	ILCGAQYRI (SEQ ID NO:116)	3.120

Table XVI

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Human HLA B7

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	180	DPMGQQGSL (SEQ ID NO:59)	240.000
2	4	DVRDLNALL (SEQ ID NO:62)	200.000
3	302	RVPGVAPTL (SEQ ID NO:195)	20.000
4	30	GAAQWAPVL (SEQ ID NO:86)	12.000
5	239	NQMNLGATL (SEQ ID NO:151)	12.000
6	130	NAPYLPACL (SEQ ID NO:144)	12.000
7	10	ALLPAVPSL (SEQ ID NO:34)	12.000
8	299	DVRRVPGVA (SEQ ID NO:63)	5.000
9	208	SCTGSQALL (SEQ ID NO:202)	4.000
10	303	VPGVAPTLV (SEQ ID NO:242)	4.000
11	18	LGGGGGCAL (SEQ ID NO:134)	4.000
12	218	RTPYSSDNL (SEQ ID NO:194)	4.000
13	207	DSCTGSQAL (SEQ ID NO:61)	4.000
14	209	CTGSQALLL (SEQ ID NO:52)	4.000
15	329	GCNKRYFKL (SEQ ID NO:90)	4.000
16	235	CMTWNQMNL (SEQ ID NO:49)	4.000
17	441	NMTKLQLAL (SEQ ID NO:149)	4.000
18	126	RMFPNAPYL (SEQ ID NO:185)	4.000
19	225	NLYQMTSQL (SEQ ID NO:147)	4.000
20	143	AIRNQGYST (SEQ ID NO:33)	3.000

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Table XVII

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Human HLA B8

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	329	GCNKRYFKL (SEQ ID NO:90)	16.000
2	4	DVRDLNALL (SEQ ID NO:62)	12.000
3	316	ETSEKRPFM (SEQ ID NO:73)	3.000
4	180	DPMGQQGSL (SEQ ID NO:59)	1.600
5	208	SCTGSQALL (SEQ ID NO:202)	0.800

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
6	130	NAPYLPSC (SEQ ID NO:144)	0.800
7	244	GATLKGVA (SEQ ID NO:88)	0.800
8	30	GAAQWAPVL (SEQ ID NO:86)	0.800
9	299	DVRRVPGVA (SEQ ID NO:63)	0.400
10	420	SCQKKFARS (SEQ ID NO:200)	0.400
11	387	TCQRKFSRS (SEQ ID NO:219)	0.400
12	225	NLYQMTSQL (SEQ ID NO:147)	0.400
13	141	QPAIRNQGY (SEQ ID NO:170)	0.400
14	10	ALLPAVPSL (SEQ ID NO:34)	0.400
15	207	DSCTGSQAL (SEQ ID NO:61)	0.400
16	384	QCKTCQRKF (SEQ ID NO:163)	0.400
17	136	SCLESQPAI (SEQ ID NO:198)	0.300
18	347	HTGEKPYQC (SEQ ID NO:112)	0.300
19	401	HTRTHTGKT (SEQ ID NO:114)	0.200
20	332	KRYFKLSHL (SEQ ID NO:127)	0.200

Table XVIII

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Human HLA B 2702

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	332	KRYFKLSHL (SEQ ID NO:127)	900.000
2	362	RRFSRSDQL (SEQ ID NO:187)	900.000
3	286	YRIHTHGVE (SEQ ID NO:252)	200.000
4	125	ARMFPNAPY (SEQ ID NO:38)	200.000
5	375	RRHTGVKPF (SEQ ID NO:188)	180.000
6	32	AQWAPVLDF (SEQ ID NO:37)	100.000
7	301	RRVPGVAPT (SEQ ID NO:189)	60.000
8	439	QRNMTKLQL (SEQ ID NO:173)	60.000
9	126	RMFPNAPYL (SEQ ID NO:185)	22.500
10	426	ARSDELVRH (SEQ ID NO:39)	20.000
11	146	NQGYSTVTF (SEQ ID NO:150)	20.000
12	144	IRNQGYSTV (SEQ ID NO:117)	20.000
13	389	QRKFSRSDH (SEQ ID NO:172)	20.000
14	263	GQSNHSTGY (SEQ ID NO:100)	20.000
15	416	CRWPSCQKK (SEQ ID NO:50)	20.000

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
16	191	QQYSVPPP (SEQ ID NO:171)	10.000
17	217	LRTPYSSDN (SEQ ID NO:140)	10.000
18	107	CRYGPFPGPP (SEQ ID NO:51)	10.000
19	98	GQFTGTAGA (SEQ ID NO:99)	10.000
20	239	NQMNLGATL (SEQ ID NO:151)	6.000

Table XIX

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Human HLA B 2705

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	332	KRYFKLSHL (SEQ ID NO:127)	30000.000
2	362	RRFSRSDQL (SEQ ID NO:187)	30000.000
3	416	CRWPSCQKK (SEQ ID NO:50)	10000.000
4	439	QRNMTKLQL (SEQ ID NO:173)	2000.000
5	286	YRIHTHGVS (SEQ ID NO:252)	1000.000
6	125	ARMFPNAPY (SEQ ID NO:38)	1000.000
7	294	FRGIQDVRR (SEQ ID NO:81)	1000.000
8	432	VRHHNMHQR (SEQ ID NO:243)	1000.000
9	169	AQFPNHSFK (SEQ ID NO:36)	1000.000
10	375	RRHTGVKPF (SEQ ID NO:188)	900.000
11	126	RMFPNAPYL (SEQ ID NO:185)	750.000
12	144	IRNQGYSTV (SEQ ID NO:117)	600.000
13	301	RRVPGVAPT (SEQ ID NO:189)	600.000
14	32	AQWAPVLDF (SEQ ID NO:37)	500.000
15	191	QQYSVPPP (SEQ ID NO:171)	300.000
16	373	HQRRHTGVK (SEQ ID NO:109)	200.000
17	426	ARSDLVHRH (SEQ ID NO:39)	200.000
18	383	FQCKTCQRK (SEQ ID NO:80)	200.000
19	239	NQMNLGATL (SEQ ID NO:151)	200.000
20	389	QRKFSRSDH (SEQ ID NO:172)	200.000

Table XX

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Human HLA B 3501

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	278	TPILCGAQY (SEQ ID NO:227)	40.000
2	141	QPAIRNQGY (SEQ ID NO:170)	40.000
3	219	TPYSSDNLY (SEQ ID NO:231)	40.000
4	327	YPGCNKRYF (SEQ ID NO:250)	20.000
5	163	TPSHHAAQF (SEQ ID NO:228)	20.000
6	180	DPMGQQGSL (SEQ ID NO:59)	20.000
7	221	YSSDNLYQM (SEQ ID NO:253)	20.000
8	26	LPVSGAAQW (SEQ ID NO:138)	10.000
9	174	HSFKHEDPM (SEQ ID NO:110)	10.000
10	82	EPHEEQCLS (SEQ ID NO:68)	6.000
11	213	QALLLRTPY (SEQ ID NO:160)	6.000
12	119	QASSGQARM (SEQ ID NO:161)	6.000
13	4	DVRDLNALL (SEQ ID NO:62)	6.000
14	40	FAPPGASAY (SEQ ID NO:74)	6.000
15	120	ASSGQARMF (SEQ ID NO:40)	5.000
16	207	DSCTGSQAL (SEQ ID NO:61)	5.000
17	303	VPGVAPTLV (SEQ ID NO:242)	4.000
18	316	ETSEKRPFM (SEQ ID NO:73)	4.000
19	152	VTFDGTSPY (SEQ ID NO:244)	4.000
20	412	KPFSCRWPS (SEQ ID NO:123)	4.000

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Table XXI

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Human HLA B 3701

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	3	SDVRDLNAL (SEQ ID NO:206)	40.000
2	273	SDNHTTPIL (SEQ ID NO:204)	40.000
3	81	AEPHEEQCL (SEQ ID NO:30)	10.000
4	298	QDVRRVPGV (SEQ ID NO:164)	8.000
5	428	SDELVRHHN (SEQ ID NO:203)	6.000

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
6	85	EEQCLSAFT (SEQ ID NO:65)	5.000
7	208	SCTGSQALL (SEQ ID NO:202)	5.000
8	4	DVRDLNALL (SEQ ID NO:62)	5.000
9	209	CTGSQALLL (SEQ ID NO:52)	5.000
10	38	LDFAPPGAS (SEQ ID NO:130)	4.000
11	223	SDNLYQMTS (SEQ ID NO:205)	4.000
12	179	EDPMGQQGS (SEQ ID NO:64)	4.000
13	206	TDSCTGSQA (SEQ ID NO:220)	4.000
14	6	RDLNALLPA (SEQ ID NO:177)	4.000
15	84	HEEQCLSAF (SEQ ID NO:107)	2.000
16	233	LECMTWNQM (SEQ ID NO:131)	2.000
17	429	DELVRHHNM (SEQ ID NO:53)	2.000
18	315	SETSEKRPF (SEQ ID NO:209)	2.000
19	349	GEKPYQCDF (SEQ ID NO:91)	2.000
20	302	RVPGVAPTL (SEQ ID NO:195)	1.500

Table XXII

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Human HLA B 3801

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	437	MHQRNMTKL (SEQ ID NO:143)	36.000
2	434	HHNMHQARN (SEQ ID NO:108)	6.000
3	372	RHQRRHTGV (SEQ ID NO:181)	6.000
4	180	DPMGQQGSL (SEQ ID NO:59)	4.000
5	433	RHHNMHQARN (SEQ ID NO:180)	3.900
6	165	SHHAAQFPN (SEQ ID NO:213)	3.900
7	202	CHTPTDSCT (SEQ ID NO:45)	3.000
8	396	DHLKTHTRT (SEQ ID NO:57)	3.000
9	161	GHTPSHHAA (SEQ ID NO:94)	3.000
10	302	RVPGVAPTL (SEQ ID NO:195)	2.600
11	417	RWPSCQKKF (SEQ ID NO:196)	2.400
12	327	YPGCNKRYF (SEQ ID NO:250)	2.400
13	208	SCTGSQALL (SEQ ID NO:202)	2.000
14	163	TPSHHAAQF (SEQ ID NO:228)	2.000
15	120	ASSGQARMF (SEQ ID NO:40)	2.000

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
16	18	LGGGGGCAL (SEQ ID NO:134)	2.000
17	177	KHEDPMGQQ (SEQ ID NO:121)	1.800
18	83	PHEEQCLSA (SEQ ID NO:154)	1.800
19	10	ALLPAVPSL (SEQ ID NO:34)	1.300
20	225	NLYQMTSQL (SEQ ID NO:147)	1.300

Table XXIII

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Human HLA B 3901

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	437	MHQRNMTKL (SEQ ID NO:143)	135.000
2	332	KRYFKLSHL (SEQ ID NO:127)	45.000
3	434	HHNMHQRNM (SEQ ID NO:108)	30.000
4	362	RRFSRSDQL (SEQ ID NO:187)	30.000
5	372	RHQRRTGV (SEQ ID NO:181)	30.000
6	10	ALLPAVPSL (SEQ ID NO:34)	9.000
7	439	QRNMTKLQL (SEQ ID NO:173)	7.500
8	390	RKFSRSDHL (SEQ ID NO:183)	6.000
9	396	DHLKTHTRT (SEQ ID NO:57)	6.000
10	239	NQMNLGATL (SEQ ID NO:151)	6.000
11	423	KKFARSDEL (SEQ ID NO:122)	6.000
12	126	RMFPNAPYL (SEQ ID NO:185)	6.000
13	225	NLYQMTSQL (SEQ ID NO:147)	6.000
14	180	DPMGQQGSL (SEQ ID NO:59)	6.000
15	144	IRNQGYSTV (SEQ ID NO:117)	5.000
16	136	SCLESQPAI (SEQ ID NO:198)	4.000
17	292	GVFRGIQDV (SEQ ID NO:103)	3.000
18	302	RVPGVAPTL (SEQ ID NO:195)	3.000
19	208	SCTGSQALL (SEQ ID NO:202)	3.000
20	207	DSCTGSQAL (SEQ ID NO:61)	3.000



Table XXIV

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Human HLA B 3902

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	239	NQMNLGATL (SEQ ID NO:151)	24.000
2	390	RKFSRSDHL (SEQ ID NO:183)	20.000
3	423	KKFARSDEL (SEQ ID NO:122)	20.000
4	32	AQWAPVLDF (SEQ ID NO:37)	5.000
5	146	NQGYSTVTF (SEQ ID NO:150)	5.000
6	130	NAPYLPSC (SEQ ID NO:144)	2.400
7	225	NLYQMTSQL (SEQ ID NO:147)	2.400
8	30	GAAQWAPVL (SEQ ID NO:86)	2.400
9	441	NMTKLQLAL (SEQ ID NO:149)	2.400
10	302	RVPGVAPTL (SEQ ID NO:195)	2.400
11	126	RMFPNAPYL (SEQ ID NO:185)	2.000
12	218	RTPYSSDNL (SEQ ID NO:194)	2.000
13	209	CTGSQALLL (SEQ ID NO:52)	2.000
14	332	KRYFKLSHL (SEQ ID NO:127)	2.000
15	180	DPMGQQGSL (SEQ ID NO:59)	2.000
16	437	MHQRNMTKL (SEQ ID NO:143)	2.000
17	207	DSCTGSQAL (SEQ ID NO:61)	2.000
18	208	SCTGSQALL (SEQ ID NO:202)	2.000
19	329	GCNKRYFKL (SEQ ID NO:90)	2.000
20	10	ALLPAVPSL (SEQ ID NO:34)	2.000

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Table XXV

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Human HLA B 4403

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	315	SETSEKRPF (SEQ ID NO:209)	80.000
2	349	GEKPYQCDF (SEQ ID NO:91)	80.000
3	84	HEEQCLSAF (SEQ ID NO:107)	60.000
4	410	SEKPFSCRW (SEQ ID NO:207)	48.000
5	429	DELVRHHNM (SEQ ID NO:53)	24.000

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
6	278	TPILCGAQY (SEQ ID NO:227)	15.000
7	141	QPAIRNQGY (SEQ ID NO:170)	9.000
8	40	FAPPGASAY (SEQ ID NO:74)	9.000
9	213	QALLLRTPY (SEQ ID NO:160)	9.000
10	318	SEKRPFMCA (SEQ ID NO:208)	8.000
11	81	AEPHEEQCL (SEQ ID NO:30)	8.000
12	152	VTFDGTSPY (SEQ ID NO:244)	4.500
13	101	TGTAGACRY (SEQ ID NO:224)	4.500
14	120	ASSGQARMF (SEQ ID NO:40)	4.500
15	261	TEGQSNHST (SEQ ID NO:221)	4.000
16	85	EEQCLSAFT (SEQ ID NO:65)	4.000
17	233	LECMTWNQM (SEQ ID NO:131)	4.000
18	104	AGACRYGPF (SEQ ID NO:31)	4.000
19	3	SDVRDLNAL (SEQ ID NO:206)	3.000
20	185	QGSLGEQQY (SEQ ID NO:166)	3.000

Table XXVI

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Human HLA B 5101

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	303	VPGVAPTLV (SEQ ID NO:242)	314.600
2	180	DPMGQQGSL (SEQ ID NO:59)	242.000
3	250	VAAGSSSSV (SEQ ID NO:236)	157.300
4	130	NAPYLPSC (SEQ ID NO:144)	50.000
5	30	GAAQWAPVL (SEQ ID NO:86)	50.000
6	20	GGGGCALPV (SEQ ID NO:92)	44.000
7	64	PPPPHSFI (SEQ ID NO:157)	40.000
8	29	SGAAQWAPV (SEQ ID NO:211)	40.000
9	18	LGGGGGAL (SEQ ID NO:134)	31.460
10	295	RGIQDVRRV (SEQ ID NO:179)	22.000
11	119	QASSGQARM (SEQ ID NO:161)	18.150
12	418	WPSCQKKFA (SEQ ID NO:246)	12.100
13	82	EPHEEQCLS (SEQ ID NO:68)	12.100
14	110	GPFPPPPS (SEQ ID NO:96)	11.000
15	272	ESDNHTTPI (SEQ ID NO:71)	8.000

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
16	306	VAPTLVRS (SEQ ID NO:237)	7.150
17	280	ILCGAQYRI (SEQ ID NO:116)	6.921
18	219	TPYSSDNLY (SEQ ID NO:231)	6.600
19	128	FPNAPYLPS (SEQ ID NO:79)	6.500
20	204	TPTDSCTGS (SEQ ID NO:230)	6.050

Table XXVII

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Human HLA B 5102

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	295	RGIQDVRRV (SEQ ID NO:179)	290.400
2	303	VPGVAPTLV (SEQ ID NO:242)	200.000
3	180	DPMGQQGSL (SEQ ID NO:59)	133.100
4	250	VAAGSSSSV (SEQ ID NO:236)	110.000
5	30	GAAQWAPVL (SEQ ID NO:86)	55.000
6	130	NAPYLPSCL (SEQ ID NO:144)	50.000
7	20	GGGGCALPV (SEQ ID NO:92)	44.000
8	29	SGAAQWAPV (SEQ ID NO:211)	44.000
9	64	PPPPHSFI (SEQ ID NO:157)	40.000
10	119	QASSGQARM (SEQ ID NO:161)	36.300
11	110	GPFGPPPPS (SEQ ID NO:96)	27.500
12	412	KPFSCRWPS (SEQ ID NO:123)	25.000
13	18	LGGGGGCAL (SEQ ID NO:134)	24.200
14	24	CALPVSGAA (SEQ ID NO:43)	16.500
15	219	TPYSSDNLY (SEQ ID NO:231)	15.000
16	292	GVFRGIQDV (SEQ ID NO:103)	14.641
17	136	SCLESQPAI (SEQ ID NO:198)	14.520
18	418	WPSCQKKFA (SEQ ID NO:246)	12.100
19	269	TGYESDNHT (SEQ ID NO:225)	11.000
20	351	KPYQCDFKD (SEQ ID NO:124)	11.000

Table XXVIII

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Human HLA B 5201

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	191	QQYSVPPP (SEQ ID NO:171)	100.000
2	32	AQWAPVLDF (SEQ ID NO:37)	30.000
3	243	LGATLKGVA (SEQ ID NO:133)	16.500
4	303	VPGVAPTLV (SEQ ID NO:242)	13.500
5	86	EQCLSAFTV (SEQ ID NO:69)	12.000
6	295	RGIQDVRRV (SEQ ID NO:179)	10.000
7	98	GQFTGTAGA (SEQ ID NO:99)	8.250
8	292	GVFRGIQDV (SEQ ID NO:103)	8.250
9	29	SGAAQWAPV (SEQ ID NO:211)	6.000
10	146	NQGYSTVTF (SEQ ID NO:150)	5.500
11	20	GGGGCALPV (SEQ ID NO:92)	5.000
12	239	NQMNLGATL (SEQ ID NO:151)	4.000
13	64	PPPPHSFI (SEQ ID NO:157)	3.600
14	273	SDNHTTPIL (SEQ ID NO:204)	3.300
15	286	YRIHTHGVF (SEQ ID NO:252)	3.000
16	269	TGYESDNHT (SEQ ID NO:225)	3.000
17	406	TGKTSEKPF (SEQ ID NO:222)	2.750
18	327	YPGCNKRYF (SEQ ID NO:250)	2.750
19	7	DLNALLPAV (SEQ ID NO:58)	2.640
20	104	AGACRYGPF (SEQ ID NO:31)	2.500

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Table XXIX

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Human HLA B 5801

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	230	TSQLECMTW (SEQ ID NO:234)	96.800
2	92	FTVHFSGQF (SEQ ID NO:85)	60.000
3	120	ASSGQARMF (SEQ ID NO:40)	40.000
4	168	AAQFPNHSF (SEQ ID NO:29)	20.000
5	408	KTSEKPFSC (SEQ ID NO:129)	12.000

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
6	394	RSDHLKTHT (SEQ ID NO:192)	9.900
7	276	HTTPILCGA (SEQ ID NO:115)	7.200
8	218	RTPYSSDNL (SEQ ID NO:194)	6.600
9	152	VTFDGTPSY (SEQ ID NO:244)	6.000
10	40	FAPPGASAY (SEQ ID NO:74)	6.000
11	213	QALLLRTPY (SEQ ID NO:160)	4.500
12	347	HTGEKPYQC (SEQ ID NO:112)	4.400
13	252	AGSSSSVKW (SEQ ID NO:32)	4.400
14	211	GSQALLLRT (SEQ ID NO:102)	4.356
15	174	HSFKHEDPM (SEQ ID NO:110)	4.000
16	317	TSEKRPFMC (SEQ ID NO:233)	4.000
17	26	LPVSGAAQW (SEQ ID NO:138)	4.000
18	289	HTHGVFRGI (SEQ ID NO:113)	3.600
19	222	SSDNLYQMT (SEQ ID NO:217)	3.300
20	96	FSGQFTGTA (SEQ ID NO:82)	3.300

Table XXX

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Human HLA CW0301

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	10	ALLPAVPSL (SEQ ID NO:34)	100.000
2	332	KRYFKLSHL (SEQ ID NO:127)	48.000
3	126	RMFPNAPYL (SEQ ID NO:185)	36.000
4	3	SDVRDLNAL (SEQ ID NO:206)	30.000
5	239	NQMNLGATL (SEQ ID NO:151)	24.000
6	225	NLYQMTSQL (SEQ ID NO:147)	24.000
7	180	DPMGQQGSL (SEQ ID NO:59)	20.000
8	362	RRFSRSDQL (SEQ ID NO:187)	12.000
9	329	GCNKRYFKL (SEQ ID NO:90)	10.000
10	286	YRIHTHGVF (SEQ ID NO:252)	10.000
11	301	RRVPGVAPT (SEQ ID NO:189)	10.000
12	24	CALPVSGAA (SEQ ID NO:43)	10.000
13	136	SCLESQPAI (SEQ ID NO:198)	7.500
14	437	MHQRNMTKL (SEQ ID NO:143)	7.200
15	390	RKFSRSDHL (SEQ ID NO:183)	6.000

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
16	423	KKFARSDEL (SEQ ID NO:122)	6.000
17	92	FTVHFSGQF (SEQ ID NO:85)	5.000
18	429	DELVRHHNM (SEQ ID NO:53)	5.000
19	130	NAPYLPSC (SEQ ID NO:144)	4.800
20	30	GAAQWAPVL (SEQ ID NO:86)	4.000

Table XXXI

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Human HLA CW0401

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	356	DFKDCERRF (SEQ ID NO:55)	120.000
2	334	YFKLSHLQM (SEQ ID NO:248)	100.000
3	180	DPMGQQGSL (SEQ ID NO:59)	88.000
4	163	TPSHHAAQF (SEQ ID NO:228)	52.800
5	327	YPGCNKRYF (SEQ ID NO:250)	40.000
6	285	QYRIHTHGV (SEQ ID NO:175)	27.500
7	424	KFARSDELV (SEQ ID NO:119)	25.000
8	326	AYPGCNKRY (SEQ ID NO:42)	25.000
9	192	QYSVPPPVY (SEQ ID NO:176)	25.000
10	417	RWPSCQKKF (SEQ ID NO:196)	22.000
11	278	TPILCGAQY (SEQ ID NO:227)	12.000
12	10	ALLPAVPSL (SEQ ID NO:34)	11.616
13	141	QPAIRNQGY (SEQ ID NO:170)	11.000
14	303	VPGVAPTLV (SEQ ID NO:242)	11.000
15	219	TPYSSDNLY (SEQ ID NO:231)	10.000
16	39	DFAPPGASA (SEQ ID NO:54)	7.920
17	99	QFTGTAGAC (SEQ ID NO:165)	6.000
18	4	DVRDLNALL (SEQ ID NO:62)	5.760
19	70	SFIKQEPSW (SEQ ID NO:210)	5.500
20	63	PPPPPHSF (SEQ ID NO:158)	5.280

Table XXXII

Results of BIMAS HLA Peptide Binding Prediction Analysis for

Binding of Human WT1 Peptides to Human HLA CW0602

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	332	KRYFKLSHL (SEQ ID NO:127)	9.680
2	239	NQMNLGATL (SEQ ID NO:151)	6.600
3	130	NAPYLPSCl (SEQ ID NO:144)	6.600
4	7	DLNALLPAV (SEQ ID NO:58)	6.000
5	441	NMTKLQLAL (SEQ ID NO:149)	6.000
6	225	NLYQMTSQL (SEQ ID NO:147)	6.000
7	4	DVRDLNALL (SEQ ID NO:62)	6.000
8	3	SDVRDLNAL (SEQ ID NO:206)	4.400
9	10	ALLPAVPSL (SEQ ID NO:34)	4.000
10	213	QALLLRTPY (SEQ ID NO:160)	3.300
11	319	EKRPFMCAY (SEQ ID NO:67)	3.000
12	30	GAAQWAPVL (SEQ ID NO:86)	2.200
13	242	NLGATLKGv (SEQ ID NO:146)	2.200
14	292	GVFRGIQDV (SEQ ID NO:103)	2.200
15	207	DSCTGSQAL (SEQ ID NO:61)	2.200
16	362	RRFSRSDQL (SEQ ID NO:187)	2.200
17	439	QRNMTKLQL (SEQ ID NO:173)	2.200
18	295	RGIQDVRRV (SEQ ID NO:179)	2.200
19	423	KKFARSDEL (SEQ ID NO:122)	2.200
20	180	DPMGQQGSL (SEQ ID NO:59)	2.200

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Table XXXIII

Results of BIMAS HLA Peptide Binding Prediction Analysis for

Binding of Human WT1 Peptides to Human HLA CW0702

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	319	EKRPFMCAY (SEQ ID NO:67)	26.880
2	326	AYPGCNKRY (SEQ ID NO:42)	24.000
3	40	FAPPGASAY (SEQ ID NO:74)	14.784
4	192	QYSVPPPVY (SEQ ID NO:176)	12.000
5	278	TPILCGAQY (SEQ ID NO:227)	12.000

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
6	219	TPYSSDNLY (SEQ ID NO:231)	12.000
7	213	QALLLRTPY (SEQ ID NO:160)	8.800
8	125	ARMFPNAPY (SEQ ID NO:38)	8.000
9	327	YPGCNKRYF (SEQ ID NO:250)	6.600
10	152	VTFDGTSPY (SEQ ID NO:244)	5.600
11	141	QPAIRNQGY (SEQ ID NO:170)	4.800
12	345	RKHTGEKPY (SEQ ID NO:184)	4.000
13	185	QGS LG EQQY (SEQ ID NO:166)	4.000
14	101	TGTAGACRY (SEQ ID NO:224)	4.000
15	375	RRHTGVKPF (SEQ ID NO:188)	4.000
16	263	GQSNHSTGY (SEQ ID NO:100)	4.000
17	163	TPSHHAAQF (SEQ ID NO:228)	3.000
18	33	QWAPVL DFA (SEQ ID NO:174)	2.688
19	130	NAPYLP SCL (SEQ ID NO:144)	2.640
20	84	HEEQCLSAF (SEQ ID NO:107)	2.400

Table XXXIV

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Mouse MHC Class I Db

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	235	CMTWNQMNL (SEQ ID NO:49)	5255.712
2	126	RMFPNAPYL (SEQ ID NO:185)	1990.800
3	221	YSSDNLYQM (SEQ ID NO:253)	930.000
4	228	QMTSQLECM (SEQ ID NO:169)	33.701
5	239	NQMNLGATL (SEQ ID NO:151)	21.470
6	441	NMTKLQLAL (SEQ ID NO:149)	19.908
7	437	MHQRNMTKL (SEQ ID NO:143)	19.837
8	136	SCLESQPAI (SEQ ID NO:198)	11.177
9	174	HSFKHEDPM (SEQ ID NO:110)	10.800
10	302	RVPGVAPT L (SEQ ID NO:195)	10.088
11	130	NAPYLP SCL (SEQ ID NO:144)	8.400
12	10	ALLPAVPSL (SEQ ID NO:34)	5.988
13	208	SCTGSQALL (SEQ ID NO:202)	4.435
14	209	CTGSQALLL (SEQ ID NO:52)	3.548
15	238	WNQMNLGAT (SEQ ID NO:245)	3.300



Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
16	218	RTPYSSDNL (SEQ ID NO:194)	3.185
17	24	CALPVSGAA (SEQ ID NO:43)	2.851
18	18	LGGGGGCAL (SEQ ID NO:134)	2.177
19	142	PAIRNQGYS (SEQ ID NO:152)	2.160
20	30	GAAQWAPVL (SEQ ID NO:86)	1.680

Table XXXV

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Mouse MHC Class I Dd

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	112	FGPPPSQA (SEQ ID NO:76)	48.000
2	122	SGQARMFPN (SEQ ID NO:212)	36.000
3	104	AGACRYGPF (SEQ ID NO:31)	30.000
4	218	RTPYSSDNL (SEQ ID NO:194)	28.800
5	130	NAPYLPSC (SEQ ID NO:144)	20.000
6	302	RVPGVAPTL (SEQ ID NO:195)	20.000
7	18	LGGGGGCAL (SEQ ID NO:134)	20.000
8	81	AEPHEEQCL (SEQ ID NO:30)	10.000
9	29	SGAAQWAPV (SEQ ID NO:211)	7.200
10	423	KKFARSD (SEQ ID NO:122)	7.200
11	295	RGIQDVRRV (SEQ ID NO:179)	7.200
12	390	RKFSRSDHL (SEQ ID NO:183)	6.000
13	332	KRYFKLSHL (SEQ ID NO:127)	6.000
14	362	RRFSRSDQL (SEQ ID NO:187)	6.000
15	417	RWPSCQKKF (SEQ ID NO:196)	6.000
16	160	YGHTPSHHA (SEQ ID NO:249)	6.000
17	20	GGGGCALPV (SEQ ID NO:92)	6.000
18	329	GCNKRYFKL (SEQ ID NO:90)	5.000
19	372	RHQRRHTGV (SEQ ID NO:181)	4.500
20	52	GGPAPPAP (SEQ ID NO:93)	4.000

Table XXXVI

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Mouse MHC Class I Kb

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	329	GCNKRYFKL (SEQ ID NO:90)	24.000
2	225	NLYQMTSQL (SEQ ID NO:147)	10.000
3	420	SCQKKFARS (SEQ ID NO:200)	3.960
4	218	RTPYSSDNL (SEQ ID NO:194)	3.630
5	437	MHQRNMTKL (SEQ ID NO:143)	3.600
6	387	TCQRKFSRS (SEQ ID NO:219)	3.600
7	302	RVPGVAPTL (SEQ ID NO:195)	3.300
8	130	NAPYLPSCSL (SEQ ID NO:144)	3.000
9	289	HTHGVFRGI (SEQ ID NO:113)	3.000
10	43	PGASAYGSL (SEQ ID NO:153)	2.400
11	155	DGTPSYGHT (SEQ ID NO:56)	2.400
12	273	SDNHHTPIL (SEQ ID NO:204)	2.200
13	126	RMFPNAPYL (SEQ ID NO:185)	2.200
14	128	FPNAPYLPS (SEQ ID NO:79)	2.000
15	3	SDVRDLNAL (SEQ ID NO:206)	1.584
16	207	DSCTGSQAL (SEQ ID NO:61)	1.584
17	332	KRYFKLSHL (SEQ ID NO:127)	1.500
18	18	LGGGGGCAL (SEQ ID NO:134)	1.320
19	233	LECMTWNQM (SEQ ID NO:131)	1.320
20	441	NMTKLQLAL (SEQ ID NO:149)	1.200

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Table XXXVII

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Mouse MHC Class I Kd

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	285	QYRIHTHGV (SEQ ID NO:175)	600.000
2	424	KFARSDELV (SEQ ID NO:119)	288.000
3	334	YFKLSHLQM (SEQ ID NO:248)	120.000

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
4	136	SCLESQPTI (SEQ ID NO:199)	115.200
5	239	NQMNLGATL (SEQ ID NO:151)	115.200
6	10	ALLPAVSSL (SEQ ID NO:35)	115.200
7	47	AYGSLGGPA (SEQ ID NO:41)	86.400
8	180	DPMGQQGSL (SEQ ID NO:59)	80.000
9	270	GYESDNHTA (SEQ ID NO:105)	72.000
10	326	AYPGCNKRY (SEQ ID NO:42)	60.000
11	192	QYSVPPPVY (SEQ ID NO:176)	60.000
12	272	ESDNHTAPI (SEQ ID NO:70)	57.600
13	289	HTHGVFRGI (SEQ ID NO:113)	57.600
14	126	DVRDLNALL (SEQ ID NO:62)	57.600
15	4	CTGSQALLL (SEQ ID NO:52)	57.600
16	208	SCTGSQALL (SEQ ID NO:202)	48.000
17	441	NMTKLQLAL (SEQ ID NO:149)	48.000
18	207	DSCTGSQAL (SEQ ID NO:61)	48.000
19	130	NAPYLPSC (SEQ ID NO:144)	48.000
20	235	CMTWNQMNL (SEQ ID NO:49)	48.000

Table XXXVIII

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Mouse MHC Class I Kk

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	81	AEPHEEQCL (SEQ ID NO:30)	40.000
2	85	EEQCLSAFT (SEQ ID NO:65)	40.000
3	429	DELVRHHNM (SEQ ID NO:53)	20.000
4	315	SETSEKRPF (SEQ ID NO:209)	20.000
5	261	TEGQSNHST (SEQ ID NO:221)	20.000
6	410	SEKPFSCRW (SEQ ID NO:207)	10.000
7	272	ESDNHTTPI (SEQ ID NO:71)	10.000
8	318	SEKRPFMCA (SEQ ID NO:208)	10.000
9	138	LESQPAIRN (SEQ ID NO:132)	10.000
10	233	LECMTWNQM (SEQ ID NO:131)	10.000
11	298	QDVRRVPGV (SEQ ID NO:164)	10.000
12	84	HEEQCLSAF (SEQ ID NO:107)	10.000
13	349	GEKPYQCDF (SEQ ID NO:91)	10.000

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
14	289	HTHGVFRGI (SEQ ID NO:113)	10.000
15	179	EDPMGQQGS (SEQ ID NO:64)	8.000
16	136	SCLESQPAI (SEQ ID NO:198)	5.000
17	280	ILCGAQYRI (SEQ ID NO:116)	5.000
18	273	SDNHTTPIL (SEQ ID NO:204)	4.000
19	428	SDELVRHHN (SEQ ID NO:203)	4.000
20	3	SDVRDLNAL (SEQ ID NO:206)	4.000

Table XXXIX

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Mouse MHC Class I Ld

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	163	TPSHHAAQF (SEQ ID NO:228)	360.000
2	327	YPGCNKRYF (SEQ ID NO:250)	300.000
3	180	DPMGQQGSL (SEQ ID NO:59)	150.000
4	26	LPVSGAAQW (SEQ ID NO:138)	93.600
5	278	TPILCGAQY (SEQ ID NO:227)	72.000
6	141	QPAIRNQGY (SEQ ID NO:170)	60.000
7	219	TPYSSDNLY (SEQ ID NO:231)	60.000
8	303	VPGVAPTLV (SEQ ID NO:242)	60.000
9	120	ASSGQARMF (SEQ ID NO:40)	50.000
10	63	PPPPPHSF (SEQ ID NO:158)	45.000
11	113	GPPPPSQAS (SEQ ID NO:97)	45.000
12	157	TPSYGHTPS (SEQ ID NO:229)	39.000
13	207	DSCTGSQAL (SEQ ID NO:61)	32.500
14	110	GPFGPPPPS (SEQ ID NO:96)	30.000
15	82	EPHEEQCLS (SEQ ID NO:68)	30.000
16	412	KPFSCRWPS (SEQ ID NO:123)	30.000
17	418	WPSCQKKFA (SEQ ID NO:246)	30.000
18	221	YSSDNLYQM (SEQ ID NO:253)	30.000
19	204	TPTDSCTGS (SEQ ID NO:230)	30.000
20	128	FPNAPYLPS (SEQ ID NO:79)	30.000

Table XL

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Human WT1 Peptides to Cattle HLA A20

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	350	EKPYQCDFK (SEQ ID NO:66)	1000.00
2	319	EKRPFMCAY (SEQ ID NO:67)	500.000
3	423	KKFARSDEL (SEQ ID NO:122)	500.000
4	345	RKHTGEKPY (SEQ ID NO:184)	500.000
5	390	RKFSRSDHL (SEQ ID NO:183)	500.000
6	137	CLESQPAIR (SEQ ID NO:47)	120.000
7	380	VKPFQCKTC (SEQ ID NO:239)	100.000
8	407	GKTSEKPFS (SEQ ID NO:95)	100.000
9	335	FKLSHLQMH (SEQ ID NO:78)	100.000
10	247	LKGVAAGSS (SEQ ID NO:135)	100.000
11	370	LKRHQRRT (SEQ ID NO:136)	100.000
12	258	VKWTEGQSN (SEQ ID NO:240)	100.000
13	398	LKTHTRTHT (SEQ ID NO:137)	100.000
14	331	NKRYFKLSH (SEQ ID NO:145)	100.000
15	357	FKDCERRFS (SEQ ID NO:77)	100.000
16	385	CKTCQRKFS (SEQ ID NO:46)	100.000
17	294	FRGIQDVRR (SEQ ID NO:81)	80.000
18	368	DQLKRHRQR (SEQ ID NO:60)	80.000
19	432	VRHHNMHQR (SEQ ID NO:243)	80.000
20	118	SQASSGQAR (SEQ ID NO:216)	80.000

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Table XLI

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Mouse WT1 Peptides to Mouse MHC Class I A 0201

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	126	RMFPNAPYL (SEQ ID NO:293)	313.968
2	187	SLGEQQYSV (SEQ ID NO:299)	285.163
3	10	ALLPAVSSL (SEQ ID NO:255)	181.794
4	225	NLYQMTSQL (SEQ ID NO:284)	68.360
5	292	GVFRGIQDV (SEQ ID NO:270)	51.790

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
6	93	TLHFSGQFT (SEQ ID NO:302)	40.986
7	191	QQYSVPPPV (SEQ ID NO:290)	22.566
8	280	ILCGAQYRI (SEQ ID NO:274)	17.736
9	441	NMTKLHVAL (SEQ ID NO:285)	15.428
10	235	CMTWNQMNL (SEQ ID NO:258)	15.428
11	7	DLNALLPAV (SEQ ID NO:261)	11.998
12	242	NLGATLKGM (SEQ ID NO:283)	11.426
13	227	YQMTSQLEC (SEQ ID NO:307)	8.573
14	239	NQMNLGATL (SEQ ID NO:286)	8.014
15	309	TLVRSASET (SEQ ID NO:303)	7.452
16	408	KTSEKPFSC (SEQ ID NO:277)	5.743
17	340	LQMHSRKHT (SEQ ID NO:280)	4.752
18	228	QMTSQLECM (SEQ ID NO:289)	4.044
19	37	VLDFAPPGA (SEQ ID NO:304)	3.378
20	302	RVSGVAPTL (SEQ ID NO:295)	1.869

Table XLII

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Mouse WT1 Peptides to Mouse MHC Class I Db

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	221	YSSDNLYQM (SEQ ID NO:308)	312.000
2	126	RMFPNAPYL (SEQ ID NO:293)	260.000
3	235	CMTWNQMNL (SEQ ID NO:258)	260.000
4	437	MHQRNMTKL (SEQ ID NO:281)	200.000
5	238	WNQMNLGAT (SEQ ID NO:305)	12.000
6	130	NAPYLPSCL (SEQ ID NO:282)	8.580
7	3	SDVRDLNAL (SEQ ID NO:298)	7.920
8	136	SCLESQPTI (SEQ ID NO:296)	7.920
9	81	AEPHEEQCL (SEQ ID NO:254)	6.600
10	10	ALLPAVSSL (SEQ ID NO:255)	6.600
11	218	RTPYSSDNL (SEQ ID NO:294)	6.000
12	441	NMTKLHVAL (SEQ ID NO:285)	3.432
13	228	QMTSQLECM (SEQ ID NO:289)	3.120
14	174	HSFKHEDPM (SEQ ID NO:272)	3.120
15	242	NLGATLKGM (SEQ ID NO:283)	2.640

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
16	261	TEGQSNHGI (SEQ ID NO:301)	2.640
17	225	NLYQMTSQL (SEQ ID NO:284)	2.640
18	207	DSCTGSQAL (SEQ ID NO:263)	2.600
19	119	QASSGQARM (SEQ ID NO:288)	2.600
20	18	LGGGGGCGL (SEQ ID NO:279)	2.600

Table XLIII

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Mouse WT1 Peptides to Mouse MHC Class I Kb

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	329	GCNKRYFKL (SEQ ID NO:268)	24.000
2	225	NLYQMTSQL (SEQ ID NO:284)	10.000
3	420	SCQKKFARS (SEQ ID NO:297)	3.960
4	218	RTPYSSDNL (SEQ ID NO:294)	3.630
5	437	MHQRNMTKL (SEQ ID NO:281)	3.600
6	387	TCQRKFSRS (SEQ ID NO:300)	3.600
7	289	HTHGVFRGI (SEQ ID NO:273)	3.000
8	130	NAPYLPSCCL (SEQ ID NO:282)	3.000
9	43	PGASAYGSL (SEQ ID NO:287)	2.400
10	155	DGAPSYGHT (SEQ ID NO:260)	2.400
11	126	RMFPNAPYL (SEQ ID NO:293)	2.200
12	128	FPNAPYLPS (SEQ ID NO:267)	2.000
13	207	DSCTGSQAL (SEQ ID NO:263)	1.584
14	3	SDVRDLNAL (SEQ ID NO:298)	1.584
15	332	KRYFKLSHL (SEQ ID NO:276)	1.500
16	233	LECMTWNQM (SEQ ID NO:278)	1.320
17	18	LGGGGGCGL (SEQ ID NO:279)	1.320
18	242	NLGATLKGM (SEQ ID NO:283)	1.200
19	123	GQARMFPN (SEQ ID NO:269)A	1.200
20	441	NMTKLHVAL (SEQ ID NO:285)	1.200

Table XLIV

Results of BIMAS HLA Peptide Binding Prediction Analysis for  
Binding of Mouse WT1 Peptides to Mouse MHC Class I Kd

Rank	Start Position	Subsequence Residue Listing	Score (Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence)
1	285	QYRIHTHGV (SEQ ID NO:291)	600.000
2	424	KFARSDELV (SEQ ID NO:275)	288.000
3	334	YFKLSHLQM (SEQ ID NO:306)	120.000
4	136	SCLESQPTI (SEQ ID NO:296)	115.200
5	239	NQMNLGATL (SEQ ID NO:286)	115.200
6	10	ALLPAVSSL (SEQ ID NO:255)	115.200
7	47	AYGSLGGPA (SEQ ID NO:256)	86.400
8	180	DPMGQQGSL (SEQ ID NO:262)	80.000
9	270	GYESDNHTA (SEQ ID NO:271)	72.000
10	192	QYSVPPPVY (SEQ ID NO:292)	60.000
11	326	AYPGCNKRY (SEQ ID NO:257)	60.000
12	289	HTHGVFRGI (SEQ ID NO:273)	57.600
13	4	DVRDLNALL (SEQ ID NO:264)	57.600
14	126	RMFPNAPYL (SEQ ID NO:293)	57.600
15	209	CTGSQALLL (SEQ ID NO:259)	48.000
16	86	EQCLSAFTL (SEQ ID NO:265)	48.000
17	302	RVSGVAPTL (SEQ ID NO:295)	48.000
18	218	RTPYSSDNL (SEQ ID NO:294)	48.000
19	272	ESDNHTAPI (SEQ ID NO:266)	48.000
20	225	NLYQMTSQL (SEQ ID NO:284)	48.000

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Table XLV

Results of TSites Peptide Binding Prediction Analysis for  
Human WT1 Peptides Capable of Eliciting a Helper T cell Response

Peptide	Sequence
p6-23	RDLNALLPAVPSLGGGG (SEQ ID NO:1)
p30-35	GAAQWA (SEQ ID NO:309)
p45-56	ASAYGSLGGPAP (SEQ ID NO:310)
p91-105	AFTVHFSGQFTGTAG (SEQ ID NO:311)
p117-139	PSQASSGQARMFPNAPYLPSCLE (SEQ ID NO:2)
p167-171	HAAQF (SEQ ID NO:312)
p202-233	CHTPTDCTGSQALLLRTPYSSDNLNLYQMTSQL (SEQ ID NO:313)
p244-262	GATLKGVAAGSSSSVKWTE (SEQ ID NO:4)



Peptide	Sequence
p287-318	RIHTHGVRGIQDVRRVPGVAPTLVRSASETS (SEQ ID NO:314)
p333-336	RYFK (SEQ ID NO:315)
p361-374	ERRFSRSDQLKRHQ (SEQ ID NO:316)
p389-410	QRKFSSRSDHLKTHTRTHTGKTS (SEQ ID NO:317)
p421-441	CQKKFARSDELVRHHNMHQRN (SEQ ID NO:318)

Certain CTL peptides (shown in Table XLVI) were selected for further study. For each peptide in Table XLVI, scores obtained using BIMAS HLA peptide binding prediction analysis are provided.

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Table XLVI

WT1 Peptide Sequences and HLA Peptide Binding Predictions

Peptide	Sequence	Comments
p329-337	GCNKRYFKL (SEQ ID NOs: 90 and 268)	Score 24,000
p225-233	NLYQMTSQL (SEQ ID NOs: 147 and 284)	binds also to class II and HLA A2, Kd, score 10,000
p235-243	CMTWNQMNL (SEQ ID NOs: 49 and 258)	binds also to HLA A2, score 5,255,712
p126-134	RMFPNAPYL (SEQ ID NOs: 185 and 293)	binds also to Kd, class II and HLA A2, score 1,990,800
p221-229	YSSDNLYQM (SEQ ID NOs: 253 and 308)	binds also to Ld, score 312,000
p228-236	QMTSQLECM (SEQ ID NOs: 169 and 289)	score 3,120
p239-247	NQMNLGATL (SEQ ID NOs: 151 and 286)	binds also to HLA A 0201, Kd, score 8,015
mouse p136-144	SCLESQPTI (SEQ ID NO:296)	binds also to Kd, 1mismatch to human
human p136-144	SCLESQPAI (SEQ ID NO:198)	score 7,920
mouse p10-18	ALLPAVSSL (SEQ ID NO:255)	binds also to Kd, HLA A2, 1 mismatch to human
human p10-18	ALLPAVPSL (SEQ ID NO:34)	score 6,600

Peptide binding to C57Bl/6 murine MHC was confirmed using the leukemia cell line RMA-S, as described by Ljunggren et al., *Nature* 346:476-480, 1990. In brief, RMA-S cells were cultured for 7 hours at 26°C in complete medium supplemented with 1% FCS. A total of  $10^6$  RMA-S cells were added into each well of a  
5 24-well plate and incubated either alone or with the designated peptide (25ug/ml) for 16 hours at 26°C and additional 3 hours at 37°C in complete medium. Cells were then washed three times and stained with fluorescein isothiocyanate-conjugated anti D<sup>b</sup> or anti-K<sup>b</sup> antibody (PharMingen, San Diego, CA). Labeled cells were washed twice, resuspended and fixed in 500ul of PBS with 1% paraformaldehyde and analyzed for  
10 fluorescence intensity in a flow cytometer (Becton-Dickinson FACSCalibur®). The percentage of increase of D<sup>b</sup> or K<sup>b</sup> molecules on the surface of the RMA-S cells was measured by increased mean fluorescent intensity of cells incubated with peptide compared with that of cells incubated in medium alone.

Mice were immunized with the peptides capable of binding to murine  
15 class I MHC. Following immunization, spleen cells were stimulated *in vitro* and tested for the ability to lyse targets incubated with WT1 peptides. CTL were evaluated with a standard chromium release assay (Chen et al., *Cancer Res.* 54:1065-1070, 1994).  $10^6$  target cells were incubated at 37°C with 150μCi of sodium <sup>51</sup>Cr for 90 minutes, in the presence or absence of specific peptides. Cells were washed three times and  
20 resuspended in RPMI with 5% fetal bovine serum. For the assay,  $10^4$  <sup>51</sup>Cr-labeled target cells were incubated with different concentrations of effector cells in a final volume of 200μl in U-bottomed 96-well plates. Supernatants were removed after 4 to 7 hours at 37°C, and the percentage specific lysis was determined by the formula:  
% specific lysis = 100 x (experimental release - spontaneous release)/(maximum  
25 release-spontaneous release).

The results, presented in Table XLVII, show that some WT1 peptides can bind to class I MHC molecules, which is essential for generating CTL. Moreover, several of the peptides were able to elicit peptide specific CTL (Figures 9A and 9B), as determined using chromium release assays. Following immunization to CTL peptides  
30 p10-18 human, p136-144 human, p136-144 mouse and p235-243, peptide specific CTL

lines were generated and clones were established. These results indicate that peptide specific CTL can kill malignant cells expressing WT1.

Table XLVII

Binding of WT1 CTL Peptides to mouse B6 class I antigens

5

Peptide	Binding Affinity to Mouse MHC Class I
Positive control	91%
negative control	0.5.-1.3%
p235-243	33.6%
p136-144 mouse	27.9%
p136-144 human	52%
p10-18: human	2.2%
p225-233	5.8%
p329-337	1.2%
p126-134	0.9%
p221-229	0.8%
p228-236	1.2%
p239-247	1%

EXAMPLE 5

USE OF A WT1 POLYPEPTIDE TO ELICIT WT1 SPECIFIC CTL IN MICE

This Example illustrates the ability of a representative WT1 polypeptide  
 10 to elicit CTL immunity capable of killing WT1 positive tumor cell lines.

P117-139, a peptide with motifs appropriate for binding to class I and  
 class II MHC, was identified as described above using TSITES and BIMAS HLA  
 peptide binding prediction analyses. Mice were immunized as described in Example 3.  
 Following immunization, spleen cells were stimulated *in vitro* and tested for the ability  
 15 to lyse targets incubated with WT1 peptides, as well as WT1 positive and negative  
 tumor cells. CTL were evaluated with a standard chromium release assay. The results,  
 presented in Figures 10A-10D, show that P117 can elicit WT1 specific CTL capable of  
 killing WT1 positive tumor cells, whereas no killing of WT1 negative cells was  
 observed. These results demonstrate that peptide specific CTL in fact kill malignant  
 20 cells expressing WT1 and that vaccine and T cell therapy are effective against  
 malignancies that express WT1.

Similar immunizations were performed using the 9-mer class I MHC binding peptides p136-144, p225-233, p235-243 as well as the 23-mer peptide p117-139. Following immunization, spleen cells were stimulated *in vitro* with each of the 4 peptides and tested for ability to lyse targets incubated with WT1 peptides. CTL were generated specific for p136-144, p235-243 and p117-139, but not for p225-233. CTL data for p235-243 and p117-139 are presented in Figures 11A and 11B. Data for peptides p136-144 and p225-233 are not depicted.

CTL lysis demands that the target WT1 peptides are endogenously processed and presented in association with tumor cell class I MHC molecules. The above WT1 peptide specific CTL were tested for ability to lyse WT1 positive versus negative tumor cell lines. CTL specific for p235-243 lysed targets incubated with the p235-243 peptides, but failed to lyse cell lines that expressed WT1 proteins (Figure 11A). By marked contrast, CTL specific for p117-139 lysed targets incubated with p117-139 peptides and also lysed malignant cells expressing WT1 (Figure 11B). As a negative control, CTL specific for p117-139 did not lyse WT1 negative EL-4 (also referred to herein as E10).

Specificity of WT1 specific lysis was confirmed by cold target inhibition (Figures 12A-12B). Effector cells were plated for various effector: target ratios in 96-well U-bottom plates. A ten-fold excess (compared to hot target) of the indicated peptide-coated target without  $^{51}\text{Cr}$  labeling was added. Finally,  $10^4$   $^{51}\text{Cr}$ -labeled target cells per well were added and the plates incubated at  $37^\circ\text{C}$  for 4 hours. The total volume per well was 200 $\mu\text{l}$ .

Lysis of TRAMP-C by p117-139 specific CTL was blocked from 58% to 36% by EL-4 incubated with the relevant peptide p117-139, but not with EL-4 incubated with an irrelevant peptide (Figure 12A). Similarly, lysis of BLK-SV40 was blocked from 18% to 0% by EL-4 incubated with the relevant peptide p117-139 (Figure 12B). Results validate that WT1 peptide specific CTL specifically kill malignant cells by recognition of processed WT1.

Several segments with putative CTL motifs are contained within p117-139. To determine the precise sequence of the CTL epitope all potential 9-mer peptides within p117-139 were synthesized (Table XLVIII). Two of these peptides (p126-134

and p130-138) were shown to bind to H-2<sup>b</sup> class I molecules (Table XLVIII). CTL generated by immunization with p117-139 lysed targets incubated with p126-134 and p130-138, but not the other 9-mer peptides within p117-139 (Figure 13A).

The p117-139 specific CTL line was restimulated with either p126-134 or p130-138. Following restimulation with p126-134 or p130-138, both T cell lines demonstrated peptide specific lysis, but only p130-138 specific CTL showed lysis of a WT1 positive tumor cell line (Figures 13B and 13C). Thus, p130-138 appears to be the naturally processed epitope.

Table XLVIII

10 Binding of WT1 CTL 9mer Peptides within p117-139 to mouse B6 class I antigens

Peptide		Binding Affinity to Mouse MHC Class I
P117-125	PSQASSGQA (SEQ ID NO:221)	2%
P118-126	SQASSGQAR (SEQ ID NO:216)	2%
P119-127	QASSGQARM (SEQ ID Nos: 161 and 288)	2%
P120-128	ASSGQARMF (SEQ ID NO:40)	1%
P121-129	SSGQARMFP (SEQ ID NO:222)	1%
P122-130	SGQARMFPN (SEQ ID NO:212)	1%
P123-131	GQARMFPNA (SEQ ID Nos: 98 and 269)	1%
P124-132	QARMFPNAP (SEQ ID NO:223)	1%
P125-133	ARMFPNAPY (SEQ ID NO:38)	1%
P126-134	RMFPNAPYL (SEQ ID NOS: 185 and 293)	79%
P127-135	MFPNAPYLP (SEQ ID NO:224)	2%
P128-136	FPNAPYLP (SEQ ID NOS: 79 and 267)	1%
P129-137	PNAPYLPSC (SEQ ID NO:225)	1%
P130-138	NAPYLPSCLE (SEQ ID NOS: 144 and 282)	79%
P131-139	APYLPSCLE (SEQ ID NO:226)	1%

#### EXAMPLE 6

##### IDENTIFICATION OF WT1 SPECIFIC mRNA IN MOUSE TUMOR CELL LINES

15 This Example illustrates the use of RT-PCR to detect WT1 specific mRNA in cells and cell lines.

Mononuclear cells were isolated by density gradient centrifugation, and were immediately frozen and stored at -80°C until analyzed by RT-PCR for the presence of WT1 specific mRNA. RT-PCR was generally performed as described by

Fraizer et al., *Blood* 86:4704-4706, 1995. Total RNA was extracted from  $10^7$  cells according to standard procedures. RNA pellets were resuspended in 25  $\mu$ L diethylpyrocarbonate treated water and used directly for reverse transcription. The zinc-finger region (exons 7 to 10) was amplified by PCR as a 330 bp mouse cDNA.

5 Amplification was performed in a thermocycler during one or, when necessary, two sequential rounds of PCR. AmpliTaq DNA Polymerase (Perkin Elmer Cetus, Norwalk, CT), 2.5 mM  $MgCl_2$  and 20 pmol of each primer in a total reaction volume of 50  $\mu$ L were used. Twenty  $\mu$ L aliquots of the PCR products were electrophoresed on 2% agarose gels stained with ethidium bromide. The gels were photographed with Polaroid film

10 (Polaroid 667, Polaroid Ltd., Hertfordshire, England). Precautions against cross contamination were taken following the recommendations of Kwok and Higuchi, *Nature* 339:237-238, 1989. Negative controls included the cDNA- and PCR-reagent mixes with water instead of cDNA in each experiment. To avoid false negatives, the presence of intact RNA and adequate cDNA generation was evaluated for each sample

15 by a control PCR using  $\beta$ -actin primers. Samples that did not amplify with these primers were excluded from analysis.

Primers for amplification of WT1 in mouse cell lines were: P115: 1458-1478: 5' CCC AGG CTG CAA TAA GAG ATA 3' (forward primer; SEQ ID NO:21); and P116: 1767-1787: 5' ATG TTG TGA TGG CGG ACC AAT 3' (reverse primer;

20 SEQ ID NO:22) (*see* Inoue et al, *Blood* 88:2267-2278, 1996; Fraizer et al., *Blood* 86:4704-4706, 1995).

Beta Actin primers used in the control reactions were: 5' GTG GGG CGC CCC AGG CAC CA 3' (sense primer; SEQ ID NO:23); and 5' GTC CTT AAT GTC ACG CAC GAT TTC 3' (antisense primer; SEQ ID NO:24)

25 Primers for use in amplifying human WT1 include: P117: 954-974: 5' GGC ATC TGA GAC CAG TGA GAA 3' (SEQ ID NO:25); and P118: 1434-1414: 5' GAG AGT CAG ACT TGA AAG CAGT 3' (SEQ ID NO:5). For nested RT-PCR, primers may be: P119: 1023-1043: 5' GCT GTC CCA CTT ACA GAT GCA 3' (SEQ ID NO:26); and P120: 1345-1365: 5' TCA AAG CGC CAG CTG GAG TTT 3' (SEQ

30 ID NO:27).

Table XLVIII shows the results of WT1 PCR analysis of mouse tumor cell lines. Within Table IV, (+++) indicates a strong WT1 PCR amplification product in the first step RT PCR, (++) indicates a WT1 amplification product that is detectable by first step WT1 RT PCR, (+) indicates a product that is detectable only in the second step of WT1 RT PCR, and (-) indicates WT1 PCR negative.

Table XLIX  
Detection of WT1 mRNA in Mouse Tumor Cell Lines

Cell Line	WT1 mRNA
K562 (human leukemia; ATCC): Positive control; (Lozzio and Lozzio, <i>Blood</i> 45:321-334, 1975)	+++
TRAMPC (SV40 transformed prostate, B6); Foster et al., <i>Cancer Res.</i> 57:3325-3330, 1997	+++
BLK-SV40 HD2 (SV40-transf. fibroblast, B6; ATCC); <i>Nature</i> 276:510-511, 1978	++
CTLL (T-cell, B6; ATCC); Gillis, <i>Nature</i> 268:154-156, 1977)	+
FM (FBL-3 subline, leukemia, B6); Glynn and Fefer, <i>Cancer Res.</i> 28:434-439, 1968	+
BALB 3T3 (ATCC); Aaroston and Todaro, <i>J. Cell. Physiol.</i> 72:141-148, 1968	+
S49.1 (Lymphoma, T-cell like, B/C; ATCC); Horibata and Harris, <i>Exp. Cell. Res.</i> 60:61, 1970	+
BNL CL.2 (embryonic liver, B/C; ATCC); <i>Nature</i> 276:510-511, 1978	+
MethA (sarcoma, B/C); Old et al., <i>Ann. NY Acad. Sci.</i> 101:80-106, 1962	-
P3.6.2.8.1 (myeloma, B/C; ATCC); <i>Proc. Natl. Acad. Sci. USA</i> 66:344, 1970	-
P2N (leukemia, DBA/2; ATCC); Melling et al., <i>J. Immunol.</i> 117:1267-1274, 1976	-
BCL1 (lymphoma, B/C; ATCC); Slavin and Strober, <i>Nature</i> 272:624-626, 1977	-
LSTRA (lymphoma, B/C); Glynn et al., <i>Cancer Res.</i> 28:434-439, 1968	-
E10/EL-4 (lymphoma, B6); Glynn et al., <i>Cancer Res.</i> 28:434-439, 1968	-

## EXAMPLE 7

## EXPRESSION IN E. COLI OF WT1 TRX FUSION CONSTRUCT

The truncated open reading frame of WT1 (WT1B) was PCR amplified with the following primers:

- 5 Forward Primer starting at amino acid 2  
 P-37 (SEQ ID NO. 342) 5' ggctccgacgtgcgggacctg 3' Tm 64°C
- Reverse Primer creating EcoRI site after stop codon  
 P-23 (SEQ ID NO. 343) 5' gaattctcaaagcgcagctggagtttgg 3' Tm 63°C

- 10 The PCR was performed under the following conditions:

- 10µl 10X Pfu buffer  
 1µl 10mM dNTPs  
 2µl 10µM each oligo  
 83µL sterile water
- 15 1.5µl Pfu DNA polymerase (Stratagene, La Jolla, CA)  
 50 ng DNA (pPDM FL WT1)
- 96°C 2 minutes  
 96°C 20 seconds 63°C 15 seconds 72°C 3 minutes x 40 cycles  
 72°C 4 minutes

- 20 The PCR product was digested with EcoRI restriction enzyme, gel purified and then cloned into pTrx 2H vector (a modified pET28 vector with a Trx fusion on the N-terminal and two His tags surrounding the Trx fusion. After the Trx fusion there exists protease cleavage sites for thrombin and enterokinase). The pTrx2H construct was digested with StuI and EcoRI restriction enzymes. The correct constructs
- 25 were confirmed by DNA sequence analysis and then transformed into BL21 (DE3) pLys S and BL21 (DE3) CodonPlus expression host cells.



## EXAMPLE 8

## EXPRESSION IN E. COLI OF WT1 A HIS TAG FUSION CONSTRUCTS

The N-terminal open reading frame of WT1 (WT1A) was PCR amplified with the following primers:

- 5 Forward Primer starting at amino acid 2  
 P-37 (SEQ ID NO. 344) 5'gggtccgacgtgcgggacctg 3' Tm 64°C  
 Reverse Primer creating EcoRI site after an artificial stop codon put after  
 amino acid 249.  
 PDM-335 (SEQ ID NO. 345) 5'gaattctcaaagcgccagctggagtttggt 3' Tm  
 10 64°C  
 The PCR was performed under the following conditions:  
 10µl 10X Pfu buffer  
 1µl 10mM dNTPs  
 2µl 10µM each oligo  
 15 83µL sterile water  
 1.5µl Pfu DNA polymerase (Stratagene, La Jolla, CA)  
 50 ng DNA (pPDM FL WT1)  
 96°C 2 minutes  
 96°C 20 seconds 63°C 15 seconds 72°C 1 minute 20 seconds x  
 20 40 cycles  
 72°C 4 minutes

The PCR product was digested with EcoRI restriction enzyme, gel purified and then cloned into pPDM, a modified pET28 vector with a His tag in frame, which had been digested with Eco72I and EcoRI restriction enzymes. The PCR product  
 25 was also transformed into pTrx 2H vector. The pTrx2H construct was digested with StuI and EcoRI restriction enzymes. The correct constructs were confirmed by DNA sequence analysis and then transformed into BL21 (DE3) pLys S and BL21 (DE3) CodonPlus expression host cells.

## EXAMPLE 9

## EXPRESSION IN E. COLI OF WT1 B HIS TAG FUSION CONSTRUCTS

The truncated open reading frame of WT1 (WT1A) was PCR amplified with the following primers:

- 5 Forward Primer starting at amino acid 250  
 PDM-346 (SEQ ID NO. 346) 5' cacagcacagggtacgagagc 3' Tm 58°C
- Reverse Primer creating EcoRI site after stop codon  
 P-23 (SEQ ID NO. 347) 5' gaattctcaaagcgccagctggagtttggt 3' Tm  
 63°C

- 10 The PCR was performed under the following conditions:

- 10µl 10X Pfu buffer  
 1µl 10mM dNTPs  
 2µl 10µM each oligo  
 83µL sterile water
- 15 1.5µl Pfu DNA polymerase (Stratagene, La Jolla, CA)  
 50 ng DNA (pPDM FL WT1)
- 96°C 2 minutes  
 96°C 20 seconds      63°C 15 seconds      72°C 1 minute 30 seconds x  
 40 cycles
- 20 72°C 4 minutes

- The PCR product was digested with EcoRI restriction enzyme, gel purified and then cloned into pPDM, a modified pET28 vector with a His tag in frame, which had been digested with Eco72I and EcoRI restriction enzymes. The PCR product was also transformed into pTrx 2H vector. The pTrx 2H construct was digested with
- 25 StuI and EcoRI restriction enzymes. The correct constructs were confirmed by DNA sequence analysis and then transformed into BL21 (DE3) pLys S and BL21 (DE3) CodonPlus expression host cells.

For Examples 7-9, the following SEQ ID NOs. are disclosed:

- SEQ ID NO. 327 is the determined cDNA sequence for Trx\_WT1\_B  
 SEQ ID NO. 328 is the determined cDNA sequence for Trx\_WT1\_A  
 SEQ ID NO. 329 is the determined cDNA sequence for Trx\_WT1  
 SEQ ID NO. 330 is the determined cDNA sequence for WT1\_A  
 5 SEQ ID NO. 331 is the determined cDNA sequence for WT1\_B  
 SEQ ID NO. 332 is the predicted amino acid sequence encoded by SEQ ID No. 327  
 SEQ ID NO. 333 is the predicted amino acid sequence encoded by SEQ ID No. 328  
 SEQ ID NO. 334 is the predicted amino acid sequence encoded by SEQ ID No. 329  
 SEQ ID NO. 335 is the predicted amino acid sequence encoded by SEQ ID No. 330  
 10 SEQ ID NO. 336 is the predicted amino acid sequence encoded by SEQ ID No. 331

#### EXAMPLE 10

##### TRUNCATED FORMS OF WT1 EXPRESSED IN E. COLI

- Three reading frames of WT1 were amplified by PCR using the  
 15 following primers:

For WT1 Tr2:

PDM-441 (SEQ ID NO. 348) 5' cacgaagaacagtgcctgagcgcattcac

3' Tm 63°C

PDM-442 (SEQ ID NO. 349) 5' ccggcgaattcatcagtataaattgtcactgc

- 20 3' TM 62°C

For WT1 Tr3:

PDM-443 (SEQ ID NO. 350) 5' caggctttgctgctgaggacgccc 3' Tm

64°C

PDM-444 (SEQ ID NO. 351) 5'

- 25 cacggagaattcatcactggtatggtttctcacc Tm 64°C

For WT1 Tr4:

PDM-445 (SEQ ID NO. 352) 5' cacagcaggaagcacactggtgagaac

3' Tm 63°C

PDM-446 (SEQ ID NO. 353) 5' ggatatctgcagaattctcaaagcgccagc

- 30 3' TM 63°C

The PCR was performed under the following conditions:

10µl 10X Pfu buffer  
 1µl 10mM dNTPs  
 2µl 10µM each oligo  
 5 83µL sterile water  
 1.5µl Pfu DNA polymerase (Stratagene, La Jolla, CA)  
 50 ng DNA (pPDM FL WT1)  
  
 96°C 2 minutes  
 96°C 20 seconds      63°C 15 seconds      72°C 30 seconds x 40  
 10 cycles  
 72°C 4 minutes

The PCR products were digested with EcoRI and cloned into pPDM His  
 (a modified pET28 vector with a His tag in frame on the 5' end) which has been  
 digested with Eco72I and EcoRI. The constructs were confirmed to be correct through  
 15 sequence analysis and transformed into BL21 pLys S and BL21 CodonPlus cells or BLR  
 pLys S and BLR CodonPlus cells.

#### EXAMPLE 11

WT1 C (amino acids 76-437) AND WT1 D (amino acids 91-437) EXPRESSION IN E. COLI  
 20 The WT1 C reading frame was amplified by PCR using the following  
 primers:

PDM-504 (SEQ ID NO. 354) 5' cactccttcatcaaacaggaac 3' Tm 61°C  
 PDM-446 (SEQ ID NO. 355) 5' ggatatctgcagaattctcaaagcgccagc 3' Tm  
 63°C

25 The PCR was performed under the following conditions:

10µl 10X Pfu buffer  
 1µl 10mM dNTPs  
 2µl 10µM each oligo  
 83µL sterile water

1.5µl Pfu DNA polymerase (Stratagene, La Jolla, CA)

50 ng DNA (pPDM FL WT1)

96°C 2 minutes

96°C 20 seconds      63°C 15 seconds      72°C 2 minutes x 40 cycles

72°C 4 minutes

The PCR product was digested with EcoRI and cloned into pPDM His which had been digested with Eco72I and EcoRI. The sequence was confirmed through sequence analysis and then transformed into BLR pLys S and BLR which is co-transformed with CodonPlus RP.

## EXAMPLE 12

### SYNTHETIC PRODUCTION OF WT1 TR-1 BY ANNEALING OVERLAPPING OLIGOS

This example was performed to determine the effect of changing proline codon usage on expression.

The following pairs of oligos were annealed:

1. PDM-505 (SEQ ID NO. 356) 5'

ggttccgacgtgcgggacctgaacgcactgctg 3'

PDM-506 (SEQ ID NO. 357) 5'

ctgccggcagcagtcggttcaggtccgcacgtcggaacc 3'

2. PDM-507 (SEQ ID NO. 358) 5'

ccggcagttccatccctgggtggcggaggctg 3'

PDM-508 (SEQ ID NO. 359) 5'

cggcagtcgcagcctccaccgccaccaggatggaa 3'

3. PDM-509 (SEQ ID NO. 360) 5'

cgcactgccggttagcgggtgcagcacagtgggctc 3'

PDM-510 (SEQ ID NO. 361) 5'

cagaactggagcccactgtgctgcaccgctaac 3'

4. PDM-511 (SEQ ID NO. 362) 5'  
cagttctggacttcgcaccgcctggatccgcatac 3'  
PDM-512 (SEQ ID NO. 363) 5'  
cagggaaccgtatgcggatgcaccaggcgggtgcgaagtc 3'
5. PDM-513 (SEQ ID NO. 364) 5'  
ggttccctgggtgggtccagcacctccgccgcaacgcc 3'  
PDM-514 (SEQ ID NO. 365) 5'  
ggcgggtggggcggttcggggcggaggtgctggaccacc 3'
6. PDM-515 (SEQ ID NO. 366) 5'  
cccaccgcctccaccgccccgcactccttcatcaaacag 3'  
PDM-516 (SEQ ID NO. 367) 5'  
ctaggttcctgtttgatgaaggagtgcggggcggtgga 3'
7. PDM-517 (SEQ ID NO. 368) 5'  
gaacctagctggggtgggtgcagaaccgcacgaagaaca 3'  
PDM-518 (SEQ ID NO. 369) 5'  
ctcaggcactgttcttcgtgcgggttctgcaccacccag 3'
8. PDM-519 (SEQ ID NO. 370) 5' gtgcctgagcgcattctgagaattctgcagat  
3'  
PDM-520 (SEQ ID NO. 371) 5'  
gtgtgatggatactgcagaattctcagaatgcg 3'

Each oligo pair was separately combined then annealed. The pairs were then ligated together and one  $\mu$ l of ligation mix was used for PCR conditions below:

- 10 $\mu$ l 10X Pfu buffer  
1 $\mu$ l 10mM dNTPs  
2 $\mu$ l 10 $\mu$ M each oligo  
83 $\mu$ L sterile water  
1.5 $\mu$ l Pfu DNA polymerase (Stratagene, La Jolla, CA)

96°C 2 minutes

96°C 20 seconds      63°C 15 seconds      72°C 30 seconds x 40  
cycles

72°C 4 minutes

- 5            The PCR product was digested with EcoRI and cloned into pPDM His which had been digested with Eco72I and EcoRI. The sequence was confirmed and then transformed into BLR pLys S and BLR which is co-transformed with CodonPlus RP.

For examples 10-12, the following SEQ ID NOs. are disclosed:

- 10   SEQ ID NO:337 is the determined cDNA sequence for WT1\_Tr1  
       SEQ ID NO:338 is the determined cDNA sequence for WT1\_Tr2  
       SEQ ID NO:339 is the determined cDNA sequence for WT1\_Tr3  
       SEQ ID NO:340 is the determined cDNA sequence for WT1\_Tr4  
       SEQ ID NO:341 is the determined cDNA sequence for WT1\_C  
 15   SEQ ID NO:342 is the predicted amino acid sequence encoded by SEQ ID NO:337  
       SEQ ID NO:343 is the predicted amino acid sequence encoded by SEQ ID NO:338  
       SEQ ID NO:344 is the predicted amino acid sequence encoded by SEQ ID NO:339  
       SEQ ID NO:345 is the predicted amino acid sequence encoded by SEQ ID NO:340  
       SEQ ID NO:346 is the predicted amino acid sequence encoded by SEQ ID NO:341

20

The WT1 C sequence represents a polynucleotide having the coding regions of TR2, TR3 and TR4.

- 25   The WT1 TR-1 synthetic sequence represents a polynucleotide in which alternative codons for proline were substituted for the native codons, producing a polynucleotide capable of expressing WT1 TR-1 in E. coli.

## EXAMPLE 13

EVALUATION OF THE SYSTEMIC HISTOPATHOLOGICAL AND TOXICOLOGICAL EFFECTS OF  
WT1 IMMUNIZATION IN MICE

5                   The purpose of this example is to analyze the immunogenicity and potential systemic histopathological and toxicological effects of WT1 protein immunization in a multiple dose titration in mice.

The experimental design for immunization of mice with WT1 protein is outlined in Table L.

10

Table LExperimental Design of WT1 Immunization in Mice

Histology Group	Corixa Group	Treatment Description	Dose Level	Total No. (Females)
1	0	No treatment	0	4
2	1.1	MPL-SE (adjuvants alone), 6x, 1 week apart	10ug	4
3	1.2	MPL-SE, 3x, 2 weeks apart	10ug	4
4	2.1	Ra12-WT1+ MPL-SE, 6x	25ug	4
5	2.2	Ra12-WT1 + MPL-SE, 3x	25ug	4
6	3.1	Ra12-WT1 + MPL-SE, 6x	100ug	4
7	3.2	Ra12-WT1 + MPL-SE, 3x	100ug	4
8	4.1	Ra12-WT1 + MPL-SE, 6x	1000ug	4
9	4.2	Ra12-WT1 + MPL-SE, 3x	1000ug	4

15                   Vaccination to WT1 protein using MPL-SE as adjuvant, in a multiple dose titration study (doses ranging from 25µg, 100µg to 1000µg WT1 protein) in female C57/B6 mice elicited a strong WT1-specific antibody response (Figure 19) and cellular T-cell responses (Figure 20).

20                   No systemic histopathological or toxicological effects of immunization with WT1 protein was observed. No histological evidence for toxicity was seen in the following tissues: adrenal gland, brain, cecum, colon, duodenum, eye, femur and



marrow, gall bladder, heart, ileum, jejunum, kidney, larynx, lacrimal gland, liver, lung, lymph node, muscle, esophagus, ovary, pancreas, parathyroid, salivary gland, sternum and marrow, spleen, stomach, thymus, trachea, thyroid, urinary bladder and uterus.

Special emphasis was put on evaluation of potential hematopoietic toxicity. The myeloid/erythroid ratio in sternum and femur marrow was normal. All evaluable blood cell counts and blood chemistry (BUN, creatinine, bilirubin, albumin, globulin) were within the normal range (Table LI).

Given that existent immunity to WT1 is present in some patients with leukemia and that vaccination to WT1 protein can elicit WT1 specific Ab and cellular T-cell responses in mice without toxicity to normal tissues, these experiments validate WT1 as a tumor/leukemia vaccine.

Table LI  
Clinical Chemistry and Hematology Analysis

**Table LI: WT1 Dose Titration Study**  
**Clinical Chemistry and Hematology Analysis**

Animal #	K/uL WBC	M/uL RBC	g/dl Hg.	% HCT	fL MCV	pg MCH	% MCHC
<b>Normal</b>	<b>5.4-16.0</b>	<b>6.7-12.5</b>	<b>10.2-16.6</b>	<b>32-54</b>	<b>31-62</b>	<b>9.2-20.8</b>	<b>22.0-35.5</b>
<b>Group 1</b>							
1 (0)	5.6	8.41	12.8	43.5	53	15.2	29.4
2 (0)	5.5	9.12	13.4	47.5	53	14.7	28.2
3 (0)	7.5	9.22	13.5	48	54	14.7	28.1
4 (0)	3.9	9.27	13.6	46	52	14.7	29.6
Mean	5.6	9.0	13.3	46.3	53.0	14.8	28.8
STD	1.5	0.4	0.4	2.0	0.8	0.3	0.8
<b>Group 2</b>							
5 (1.5)	6.6	9	13.1	46	54	14.5	28.5
6 (1.6)	5.2	8.58	12.6	44	53	14.7	28.6
7 (1.7)	7.8	9.21	13.6	46	53	14.7	29.6
8 (1.8)	6.3	NA	NA	41	NA	NA	NA
Mean	6.5	8.9	13.1	44.3	53.3	14.6	28.9
STD	1.1	0.3	0.5	2.4	0.6	0.1	0.6
<b>Group 3</b>							
9 (2.5)	8.3	9.16	13.6	50.3	55	14.9	27.1
10 (2.6)	5	8.78	13	44.2	50	14.8	29.3

**Table LI: WT1 Dose Titration Study**  
**Clinical Chemistry and Hematology Analysis**

Animal #	K/uL WBC	M/uL RBC	g/dl Hg.	% HCT	fL MCV	pg MCH	% MCHC
<b>Normal</b>	<b>5.4-16.0</b>	<b>6.7-12.5</b>	<b>10.2-16.6</b>	<b>32-54</b>	<b>31-62</b>	<b>9.2-20.8</b>	<b>22.0-35.5</b>
<b>11 (2.7)</b>	4	8.94	13.2	48.3	54	14.7	27.3
<b>12 (2.8)</b>	8.2	NA	NA	41	NA	NA	NA
<b>Mean</b>	6.4	9.0	13.3	46.0	53.0	14.8	27.9
<b>STD</b>	2.2	0.2	0.3	4.2	2.6	0.1	1.2
<b>Group 4</b>							
<b>13 (3.5)</b>	6.1	8.82	13.1	46	54	14.9	28.5
<b>14 (3.6)</b>	6.1	8.64	12.9	46	54	15	28
<b>15 (3.7)</b>	9.3	8.93	13.2	48	55	14.8	27.5
<b>16 (3.8)</b>	4.8	8.19	12.6	44	55	15.3	28.6
<b>Mean</b>	6.6	8.6	13.0	46.0	54.5	15.0	28.2
<b>STD</b>	1.9	0.3	0.3	1.6	0.6	0.2	0.5
<b>Group 5</b>							
<b>17 (4.5)</b>	3.1	8.48	12.6	46	54	14.9	27.5
<b>18 (4.6)</b>	5.7	9.12	13.7	48	54	15	28.5
<b>19 (4.7)</b>	5.3	8.58	13	44.5	55	15.2	29.2
<b>20 (4.8)</b>	5.3	NA	NA	40	NA	NA	NA
<b>Mean</b>	4.9	8.7	13.1	44.6	54.3	15.0	28.4
<b>STD</b>	1.2	0.3	0.6	3.4	0.6	0.2	0.9
<b>Group 6</b>							
<b>21 (1.1)</b>	3.5	9.36	13.5	37.6	40	14.4	35.9
<b>22 (1.2)</b>	6.9	8.93	13.6	37.3	42	15.3	36.6
<b>23 (1.3)</b>	3.6	8.3	12.5	35.3	43	15.1	35.5
<b>24 (1.4)</b>	NA	NA	NA	NA	NA	NA	NA
<b>Mean</b>	4.7	8.9	13.2	36.7	41.7	14.9	36.0
<b>STD</b>	1.9	0.5	0.6	1.3	1.5	0.5	0.6
<b>Group 7</b>							
<b>25 (2.1)</b>	4	NA	NA	40	NA	NA	NA
<b>26 (2.2)</b>	7.4	9.12	13.2	38.5	42	14.5	34.3
<b>27 (2.3)</b>	4.5	8.19	12.1	34.5	42	14.8	35.1
<b>28 (2.4)</b>	5.8	8.25	12.3	34.1	41	14.9	36.1
<b>Mean</b>	5.4	8.5	12.5	36.8	41.7	14.7	35.2
<b>STD</b>	1.5	0.5	0.6	2.9	0.6	0.2	0.9
<b>Group 8</b>							
<b>29 (3.1)</b>	5.1	8.53	12.6	34.9	41	14.7	36
<b>30 (3.2)</b>	7.6	8.42	13	36.1	43	15.4	35.9
<b>31 (3.3)</b>	3.4	8.45	12.6	34.9	41	14.9	36.1
<b>32 (3.4)</b>	6.1	8.11	12.3	34.8	43	15.2	35.5
<b>Mean</b>	5.6	8.4	12.6	35.2	42.0	15.1	35.9
<b>STD</b>	1.8	0.2	0.3	0.6	1.2	0.3	0.3

**Table LI: WT1 Dose Titration Study**  
**Clinical Chemistry and Hematology Analysis**

Animal #	K/uL WBC	M/uL RBC	g/dl Hg.	% HCT	fL MCV	pg MCH	% MCHC
Normal	5.4-16.0	6.7-12.5	10.2-16.6	32-54	31-62	9.2-20.8	22.0-35.5
Group 9							
33 (4.1)	NA	NA	NA	NA	NA	NA	NA
34 (4.2)	4.5	8.63	12.8	36.2	42	14.8	35.2
35 (4.3)	3.9	8.85	13	36.6	41	14.7	35.6
36 (4.4)	4.7	8.14	12.3	33.8	42	15.1	36.3
Mean	4.4	8.5	12.7	35.5	41.7	14.9	35.7
STD	0.4	0.4	0.4	1.5	0.6	0.2	0.6

**Table LI (cont'd): WT1 Dose Titration Study**  
**Clinical Chemistry and Hematology Analysis**

Animal #	yes/no Plt. clump	K/uL Platelets	Abs. Baso	Abs. Eos	Abs. Bands	Abs. Polys	Abs. Lymph	Abs. Mono
Normal	no	150-1500	0.0-0.15	0.0-0.51	0.0-0.32	8.0-42.9	8.0-18.0	0.0-1.5
Group 1			K/uL	K/uL	K/uL	K/uL	K/uL	K/uL
1 (0)	yes	726	0	56	0	336	5208	0
2 (0)	no	860	0	0	0	55	5445	0
3 (0)	no	875	0	375	0	525	6525	75
4 (0)	yes	902	0	0	0	156	3744	0
Mean		840.8	0.0	107.8	0.0	268.0	5230.5	18.8
STD		78.4	0.0	180.1	0.0	207.0	1144.8	37.5
Group 2								
5 (1.5)	no	1193	0	132	0	792	5214	462
6 (1.6)	no	1166	0	52	0	624	4472	52
7 (1.7)	no	1087	0	234	0	1170	6396	0
8 (1.8)	yes	NA	0	126	0	126	5922	126
Mean		1148.7	0.0	136.0	0.0	678.0	5501.0	160.0
STD		55.1	0.0	74.8	0.0	433.1	840.5	207.9
Group 3								
9 (2.5)	no	705	0	166	0	664	7387	83
10 (2.6)	no	1140	0	150	0	500	4350	0
11 (2.7)	no	952	0	120	0	680	3200	0
12 (2.8)	yes	NA	0	164	0	656	7216	164
Mean		932.3	0.0	150.0	0.0	625.0	5538.3	61.8
STD		218.2	0.0	21.2	0.0	83.9	2090.6	78.6
Group 4								
13 (3.5)	no	785	0	488	0	732	4636	244
14 (3.6)	yes	973	0	0	0	488	5307	305

15 (3.7)	yes	939	0	465	0	558	7812	465
16 (3.8)	yes	1622	0	192	0	480	4080	48
Mean		1079.8	0.0	286.3	0.0	564.5	5458.8	265.5
STD		370.6	0.0	233.4	0.0	117.0	1647.1	172.4
<b>Group 5</b>								
17 (4.5)	no	892	0	31	0	620	2449	0
18 (4.6)	yes	966	57	114	0	855	4674	0
19 (4.7)	yes	883	0	53	0	742	4452	53
20 (4.8)	yes	NA	0	106	0	53	5141	0
Mean		913.7	14.3	76.0	0.0	567.5	4179.0	13.3
STD		45.5	28.5	40.4	0.0	356.2	1188.5	26.5

**Table LI (cont'd): WT1 Dose Titration Study  
Clinical Chemistry and Hematology Analysis**

Animal #	yes/no Plt. clump	K/uL Platelets	Abs. Baso	Abs. Eos	Abs. Bands	Abs. Polys	Abs. Lymph	Abs. Mono
Normal	no	150-1500	0.0-0.15	0.0-0.51	0.0-0.32	8.0-42.9	8.0-18.0	0.0-1.5
<b>Group 6</b>								
21 (1.1)	yes	784	0	35	0	385	2870	210
22 (1.2)	yes	806	0	69	0	207	6486	138
23 (1.3)	yes	790	0	180	0	396	2988	36
24 (1.4)	NA	NA	NA	NA	NA	NA	NA	NA
Mean		793.3	0.0	94.7	0.0	329.3	4114.7	128.0
STD		11.4	0.0	75.8	0.0	106.1	2054.5	87.4
<b>Group 7</b>								
25 (2.1)	yes	NA	0	80	0	200	3720	0
26 (2.2)	yes	753	0	0	0	518	6734	148
27 (2.3)	yes	725	0	90	0	225	4140	45
28 (2.4)	yes	792	0	232	0	754	4814	0
Mean		756.7	0.0	100.5	0.0	424.3	4852.0	48.3
STD		33.7	0.0	96.5	0.0	263.0	1333.1	69.8
<b>Group 8</b>								
29 (3.1)	yes	784	0	153	0	561	4233	153
30 (3.2)	yes	512	0	152	0	304	6992	152
31 (3.3)	yes	701	0	0	0	238	3094	68
32 (3.4)	yes	631	0	305	0	305	5368	122
Mean		657.0	0.0	152.5	0.0	352.0	4921.8	123.8
STD		115.1	0.0	124.5	0.0	142.8	1663.3	39.9
<b>Group 9</b>								
33 (4.1)	NA	NA	NA	NA	NA	NA	NA	NA
34 (4.2)	yes	724	0	125	0	540	3780	45
35 (4.3)	yes	758	0	117	0	429	3315	39
36 (4.4)	yes	808	0	47	0	329	4089	235
Mean		763.3	0.0	96.3	0.0	432.7	3728.0	106.3
STD		42.3	0.0	42.9	0.0	105.5	389.6	111.5

**Table LI (cont'd): WT1 Dose Titration Study  
Clinical Chemistry and Hematology Analysis**

<b>Animal #</b>	<b>mg/dl BUN</b>	<b>mg/dl Creatinine</b>	<b>g/dl T. protein</b>	<b>g/dl Albumin</b>	<b>g/dl Globulin</b>	<b>mg/dl T. Bilirubin</b>
<b>Normal</b>	<b>13.9-28.3</b>	<b>0.3-1.0</b>	<b>4.0-8.6</b>	<b>2.5-4.8</b>	<b>1.5-3.8</b>	<b>0.10-0.90</b>
<b>Group 1</b>						
<b>1 (0)</b>	NA	NA	NA	NA	NA	NA
<b>2 (0)</b>	28	0.5	4.9	3.7	1.2	0.3
<b>3 (0)</b>	25	0.5	4.9	3.8	1.1	0.2
<b>4 (0)</b>	27	0.5	4.7	3.7	1	0.2
<b>Mean</b>	26.7	0.5	4.8	3.7	1.1	0.2
<b>STD</b>	1.5	0.0	0.1	0.1	0.1	0.1
<b>Group 2</b>						
<b>5 (1.5)</b>	34	0.5	4.6	3.6	1	0.2
<b>6 (1.6)</b>	31	0.4	4.6	3.3	1.3	0.2
<b>7 (1.7)</b>	34	0.6	4.9	4	0.9	0.3
<b>8 (1.8)</b>	NA	NA	NA	NA	NA	NA
<b>Mean</b>	33.0	0.5	4.7	3.6	1.1	0.2
<b>STD</b>	1.7	0.1	0.2	0.4	0.2	0.1
<b>Group 3</b>						
<b>9 (2.5)</b>	NA	NA	NA	NA	NA	NA
<b>10 (2.6)</b>	33	0.5	4.6	3.6	1	0.3
<b>11 (2.7)</b>	NA	NA	NA	NA	NA	NA
<b>12 (2.8)</b>	31	0.5	4.8	3.7	1.1	0.2
<b>Mean</b>	32.0	0.5	4.7	3.7	1.1	0.3
<b>STD</b>	1.4	0.0	0.1	0.1	0.1	0.1
<b>Group 4</b>						
<b>13 (3.5)</b>	32	0.7	4.6	3.4	1.2	0.2
<b>14 (3.6)</b>	34	0.4	4.8	3.8	1	0.2
<b>15 (3.7)</b>	30	0.4	4.7	3.4	1.3	0.2
<b>16 (3.8)</b>	24	0.3	5.1	3.8	1.3	0.2
<b>Mean</b>	30.0	0.5	4.8	3.6	1.2	0.2
<b>STD</b>	4.3	0.2	0.2	0.2	0.1	0.0
<b>Group 5</b>						
<b>17 (4.5)</b>	22	0.4	4.6	3.3	1.3	0.2
<b>18 (4.6)</b>	31	0.5	4.9	3.7	1.2	0.2
<b>19 (4.7)</b>	23	0.6	4.8	3.6	1.2	0.2
<b>20 (4.8)</b>	28	0.5	4.5	3.4	1.1	0.2
<b>Mean</b>	26.0	0.5	4.7	3.5	1.2	0.2
<b>STD</b>	4.2	0.1	0.2	0.2	0.1	0.0

**Table LI (cont'd): WT1 Dose Titration Study  
Clinical Chemistry and Hematology Analysis**

<b>Animal #</b>	<b>mg/dl BUN</b>	<b>mg/dl Creatinine</b>	<b>g/dl T. protein</b>	<b>g/dl Albumin</b>	<b>g/dl Globulin</b>	<b>mg/dl T. Bilirubin</b>
<b>Normal</b>	<b>13.9-28.3</b>	<b>0.3-1.0</b>	<b>4.0-8.6</b>	<b>2.5-4.8</b>	<b>1.5-3.8</b>	<b>0.10-0.90</b>
<b>Group 6</b>						
<b>21 (1.1)</b>	28	0.3	5.1	3.4	1.7	0.2
<b>22 (1.2)</b>	36	0.3	5.1	3.8	1.3	0.2
<b>23 (1.3)</b>	32	0.4	4.9	3.5	1.4	0.1
<b>24 (1.4)</b>	NA	NA	NA	NA	NA	NA
<b>Mean</b>	32.0	0.3	5.0	3.6	1.5	0.2
<b>STD</b>	4.0	0.1	0.1	0.2	0.2	0.1
<b>Group 7</b>						
<b>25 (2.1)</b>	32	0.2	5	3.4	1.6	0.2
<b>26 (2.2)</b>	24	0.3	4.2	2.8	1.4	0.1
<b>27 (2.3)</b>	28	0.3	4.8	3.2	1.6	0.2
<b>28 (2.4)</b>	27	0.3	5	3.4	1.6	0.1
<b>Mean</b>	27.8	0.3	4.8	3.2	1.6	0.2
<b>STD</b>	3.3	0.0	0.4	0.3	0.1	0.1
<b>Group 8</b>						
<b>29 (3.1)</b>	32	0.3	4.9	3.3	1.6	0.2
<b>30 (3.2)</b>	NA	NA	NA	NA	NA	NA
<b>31 (3.3)</b>	18	0.3	4.8	3.1	1.7	0.2
<b>32 (3.4)</b>	26	0.2	4.2	2.9	1.3	0
<b>Mean</b>	25.3	0.3	4.6	3.1	1.5	0.1
<b>STD</b>	7.0	0.1	0.4	0.2	0.2	0.1
<b>Group 9</b>						
<b>33 (4.1)</b>	25	0.2	4.1	2.7	1.4	0.3
<b>34 (4.2)</b>	NA	NA	NA	NA	NA	NA
<b>35 (4.3)</b>	23	0.2	4.7	3.1	1.6	0.2
<b>36 (4.4)</b>	29	0.3	4.7	3.2	1.5	0.3
<b>Mean</b>	25.7	0.2	4.5	3.0	1.5	0.3
<b>STD</b>	3.1	0.1	0.3	0.3	0.1	0.1

Abbreviations: WBC: white blood cells; RBC: red blood cells; Hg.: hemoglobin; HCT: hematocrit ; MCV: Mean corpuscular volume; MCH: mean corpuscular hemoglobin;  
 5 MCHC: mean corpuscular hemoglobin concentration; Plt.: platelets; Abs.: Absolute; Baso: basophils; Eos: eosinophils; Abs. Bands: immature neutrophils ; Polys: polymorphonuclear cells; Lymph: lymphocytes; Mono: monocytes; BUN: blood urea nitrogen

## EXAMPLE 14

ELICITATION OF HUMAN WT1-SPECIFIC T-CELL RESPONSES BY WHOLE GENE IN VITRO  
PRIMING

This example demonstrates that WT1 specific T-cell responses can be  
5 generated from the blood of normal individuals.

Dendritic cells (DC) were differentiated from monocyte cultures derived from PBMC of normal donors by growth for 4-10 days in RPMI medium containing 10% human serum, 50 ng/ml GM-CSF and 30 ng/ml IL-4. Following culture, DC were infected 16 hours with recombinant WT1-expressing vaccinia virus at an M.O.I. of 5, or  
10 for 3 days with recombinant WT1-expressing adenovirus at an M.O.I. of 10 (Figures 21 and 22). Vaccinia virus was inactivated by U.V. irradiation. CD8+ T-cells were isolated by positive selection using magnetic beads, and priming cultures were initiated in 96-well plates. Cultures were restimulated every 7-10 days using autologous dendritic cells adeno or vaccinia infected to express WT1. Following 3-6 stimulation  
15 cycles, CD8+ lines could be identified that specifically produced interferon-gamma when stimulated with autologous-WT1-expressing dendritic cells or fibroblasts. The WT1-specific activity of these lines could be maintained following additional stimulation cycles. These lines were demonstrated to specifically recognize adeno or vaccinia WT1 infected autologous dendritic cells but not adeno or vaccinia EGFP-  
20 infected autologous dendritic cells by Elispot assays (Figure 23).

## EXAMPLE 15

FORMULATION OF RA12-WT1 FOR INJECTION: USE OF EXCIPIENTS TO STABILIZE  
LYOPHILIZED PRODUCT

25 This example describes the formulation that allows the complete solubilization of lyophilized Ra12-WT1.

The following formulation allowed for the recombinant protein Ra12-WT1 to be dissolved into an aqueous medium after being lyophilized to dryness:

Recombinant Ra12-WT1 concentration: 0.5 – 1.0 mg/ml; Buffer: 10-20  
30 mM Ethanolamine, pH 10.0; 1.0 – 5.0 mM Cysteine; 0.05 % Tween-80 (Polysorbate-80); Sugar: 10% Trehalose (T5251, Sigma, MO) 10% Maltose (M9171, Sigma, MO)

10% Sucrose (S7903, Sigma, MO) 10% Fructose (F2543, Sigma, MO) 10% Glucose (G7528, Sigma, MO).

The lyophilized protein with the sugar excipient was found to dissolve significantly more than without the sugar excipient. Analysis by coomassie stained SDS-PAGE showed no signs of remaining solids in the dissolved material.

## EXAMPLE 16

### FORMULATION OF A WT1 PROTEIN VACCINE

This example describes the induction of WT1-specific immune responses following immunization with WT1 protein and 2 different adjuvant formulations.

According to this example, WT1 protein in combination with MPL-SE induces a strong Ab and Interferon- $\gamma$  (IFN- $\gamma$ ) response to WT1. Described in detail below are the methods used to induce WT1 specific immune responses following WT1 protein immunization using MPL-SE or Enhanzyn as adjuvant in C57/B6 mice.

C57BL/6 mice were immunized with 20  $\mu$ g rRa12-WT1 combined with either MPL-SE or Enhanzyn adjuvants. One group of control mice was immunized with rRa12-WT1 without adjuvant and one group was immunized with saline alone. Three intramuscular (IM) immunizations were given, three weeks apart. Spleens and sera were harvested 2 weeks post-final immunization. Sera were analyzed for antibody responses by ELISA on plates coated with Ra12-WT1 fusion, Ra12 or WT1TRX. Similar levels of IgG2a and IgG1 antibody titers were observed in mice immunized with Ra12-WT1+MPL-SE and Ra12-WT1+Enhanzyn. Mice immunized with rRa12-WT1 without adjuvant showed lower levels of IgG2a antibodies.

CD4 responses were assessed by measuring Interferon- $\gamma$  production following stimulation of splenocytes *in vitro* with rRa12-WT1, rRa12 or with WT1 peptides p6, p117 and p287. Both adjuvants improved the CD4 responses over mice immunized with rRA12-WT1 alone. Additionally, the results indicate that rRA12-WT1+MPL-SE induced a stronger CD4 response than did rRA12-WT1+Enhanzyn. IFN- $\gamma$  OD readings ranged from 1.4-1.6 in the mice immunized with rRA12-WT1+MPL-SE as compared to 1-1.2 in the mice immunized with rRA12-



WT1+Enhazyn. Peptide responses were only observed against p117, and then only in mice immunized with rRA12-WT1+MPL-SE. Strong IFN- $\gamma$  responses to the positive control, ConA, were observed in all mice. Only responses to ConA were observed in the negative control mice immunized with saline indicating that the responses were specific to rRA12-WT1.

#### EXAMPLE 17

##### CONSTRUCTION OF A RANDOMLY MUTATED WT1 LIBRARY

The nucleic acid sequence of human WT1 was randomly mutated using a polymerase chain reaction method in the presence of 8-oxo dGTP and dPTP (journal of Molecular Biology 1996; 255:589-603). The complete unspliced human WT1 gene is disclosed in SEQ ID NO:380 and the corresponding protein sequence is set forth in SEQ ID NO:404. A splice variant of WT1 was used as a template for the PCR reactions and is disclosed in SEQ ID NOs:381 (DNA) and 408 (protein). Conditions were selected so that the frequency of nucleic acid alterations led to a targeted change in the amino acid sequence, usually 5-30% of the PCR product. The mutated PCR product was then amplified in the absence of the nucleotide analogues using the four normal dNTPs. This PCR product was subcloned into mammalian expression vectors and viral vectors for immunization. This library, therefore, contains a mixed population of randomly mutated WT1 clones. Several clones were selected and sequenced. The mutated WT1 variant DNA sequences are disclosed in SEQ ID NOs:377-379 and the predicted amino acid sequences of the variants are set forth in SEQ ID NOs:405-407. These altered sequences, and others from the library, can be used as immunogens to induce stronger T cell responses against WT1 protein in cancer cells.

#### EXAMPLE 18

##### CONSTRUCTION OF WT1-LAMP FUSIONS

A tripartite fusion was constructed using the polymerase chain reaction and synthetic oligonucleotides containing the desired junctions of human lysosomal

associated membrane protein-1 (LAMP-1) and a splice variant of the human WT1 sequence. The splice variant of WT1 and the LAMP-1 sequence used for these fusions are disclosed in SEQ ID NOs:381 and 383. Specifically, the signal peptide of LAMP-1 (base pairs 1-87 of LAMP) was fused to the 5-prime end of the human WT1 open  
5 reading frame (1,290 base pairs in length), then the transmembrane and cytoplasmic domain of LAMP-1 (base pairs 1161 to 1281 of LAMP) was fused to the 3-prime end of the WT1 sequence. The sequence of the resulting WT1-LAMP construct is set forth in SEQ ID NO:382 (DNA) and SEQ ID NO:409 (protein). The construct was designed so that when it is expressed in eukaryotic cells, the signal peptide directs the protein to the  
10 endoplasmic reticulum (ER) where the localization signals in the transmembrane and cytoplasmic domain of LAMP-1 direct transport of the fusion protein to the lysosomal location where peptides are loaded on to Class II MHC molecules.

#### EXAMPLE 19

##### 15 CONSTRUCTION OF WT1-UBIQUITIN FUSIONS FOR ENHANCED MHC CLASS I PRESENTATION

The human ubiquitin open reading frame (SEQ ID NO:384) was mutated such that the nucleotides encoding the last amino acid encode an alanine instead of a glycine. This mutated open reading frame was cloned in frame just upstream of the first  
20 codon of a splice variant of human WT1 (SEQ ID NOs:381 and 408, DNA and protein, respectively). The G->A mutation prevents co-translational cleavage of the nascent protein by the proteases that normally process poly-ubiquitin during translation. The DNA and predicted amino acid sequence for the resulting construct are set forth in SEQ ID NOs:385 and 410, respectively. The resulting protein demonstrated decreased  
25 cellular cytotoxicity when it was expressed in human cells. Whereas it was not possible to generate stable lines expressing native WT1, cell lines expressing the fusion protein were readily obtained. The resulting protein is predicted to be targeted to the proteasome by virtue of the added ubiquitin molecule. This should result in more efficient recognition of the protein by WT1 specific CD8+ T cells.

30

## EXAMPLE 20

## CONSTRUCTION OF AN ADENOVIRUS VECTOR EXPRESSING HUMAN WT1

A splice variant of human WT1 (SEQ ID NO:381) was cloned into an E1  
5 and E3 deleted adenovirus serotype 5 vector. The expression of the WT1 gene is  
controlled by the CMV promoter mediating high levels of WT1 protein expression.  
Infection of human cells with this reagent leads to a high level of expression of the WT1  
protein. The antigenic nature of the adenoviral proteins introduced into the host cell  
during and produced at low levels subsequent to infection can act to increase immune  
10 surveillance and immune recognition of WT1 as an immunological target. This vector  
can be also used to generate immune responses against the WT1 protein when  
innoculated into human subjects. If these subjects are positive for WT1 expressing  
tumor cells the immune response could have a therapeutic or curative effect on the  
course of the disease.

15

## EXAMPLE 21

## CONSTRUCTION OF A VACCINIA VIRUS VECTOR EXPRESSING HUMAN WT1

A splice variant of the full length human WT1 gene (SEQ ID NO:381)  
20 was cloned into the thymidine kinase locus of the Western Reserve strain of the  
vaccinia virus using the pSC11 shuttle vector. The WT1 gene is under the control of a  
hybrid vaccinia virus promoter that mediates gene expression throughout the course of  
vaccinia virus infection. This reagent can be used to express the WT1 protein in human  
cells in vivo or in vitro. WT1 is a self protein that is overexpressed on some human  
25 tumor cells. Thus, immunological responses to WT1 delivered as a protein are unlikely  
to lead to Major Histocompatibility Class I (MHC class I)-mediated recognition of  
WT1. However, expression of the protein in the intracellular compartment by the  
vaccinia virus vector will allow high level MHC class I presentation and recognition of  
the WT1 protein by CD8+ T cells. Expression of the WT1 protein by the vaccinia virus  
30 vector will also lead to presentation of WT1 peptides in the context of MHC class II and  
thus to recognition by CD4+ T cells.

The uses of this invention include its use as a cancer vaccine. Immunization of human subjects bearing WT1 positive tumors could lead to a therapeutic or curative response. The expression of WT1 within the cell will lead to recognition of the protein by both CD4 and CD8 positive T cells.

5

## EXAMPLE 22

## GENERATION OF WT1-SPECIFIC CD8+ T-CELL CLONES USING WHOLE GENE PRIMING

Dendritic cells (DC) were differentiated from monocyte cultures derived from PBMC of normal donors by growth for 4-6 days in RPMI medium containing 10% human serum, 50 ng/ml GM-CSF and 30 ng/ml IL-4. Following culture, DC were infected 16 hours with recombinant WT1-expressing vaccinia virus (described in Example 21) at a multiplicity of infection (MOI) of 5 or for 3 days with recombinant WT1-expressing adenovirus at an MOI of 10. Vaccinia virus was inactivated by U.V. irradiation. CD8+ T-cells were isolated by negative depletion using magnetic beads, and priming cultures were initiated in 96-well plates. Cultures were restimulated every 7-10 days using autologous dendritic cells infected with adeno or vaccinia virus engineered to express WT1. Following 4-5 stimulation cycles, CD8+ T-cell lines could be identified that specifically produced interferon-gamma when stimulated with autologous-WT1 expressing dendritic cells or fibroblasts. These lines were cloned and demonstrated to specifically recognize WT1 transduced autologous fibroblasts but not EGFP transduced fibroblasts by Elispot assays.

The Wilms' tumor (WT1) gene participates in leukemogenesis and is overexpressed in most human leukemias as well as in several solid tumors. Previous studies in humans have demonstrated the presence of WT1 specific antibody (Ab) responses in 16/63 (25%) of AML and in 15/81 (19%) of CML patients studied. Previous studies in mice have shown that WT1 peptide based vaccines elicit WT1 specific Ab, Th and CTL responses. The use of peptides as vaccines in humans is limited by their HLA restriction and the tendency to elicit peptide specific responses and only in a minority of patients tumor specific CTL. The advantages of whole gene immunization are that several helper and CTL epitopes can be included in a single

vaccine, thus not restricting the vaccine to specific HLA types. The data disclosed herein demonstrate the induction of WT1 specific immune responses using whole gene in vitro priming, and that WT1 specific CD8+ T-cell clones can be generated. Given that existent immunity to WT1 is present in some patients with leukemia and that murine and human WT1 are 96% identical at the amino acid level and vaccination to WT1 protein, DNA or peptides can elicit WT1 specific Ab, and cellular T-cell responses in mice without toxicity to normal tissues in mice, these human in vitro priming experiments provide further validation of WT1 as a tumor/leukemia vaccine. Furthermore, the ability to generate WT1 specific CD8+ T-cell clones may lead to the treatment of malignancies associated with WT1 overexpression using genetically engineered T-cells.

### EXAMPLE 23

#### RECOMBINANT CONSTRUCTS FOR CLINICAL MANUFACTURING OF WT1

Five constructs were made as described in detail below, for the production of clinical grade WT1.

#### **Design of Ra12/WT-E (SEQ ID NOs:388 (cDNA) and 391 (protein)) and WT-1 E (SEQ ID NOs:386 (cDNA) and 395 (protein)) with No His tag:**

The WT-1 E reading frame was PCR amplified with the following primers for the non-His non fusion construct:

PDM-780 (SEQ ID NO:396) 5' gacgaaagcatatgcactcctcatcaaac 3' Tm 60°C

PDM-779 (SEQ ID NO:397) 5' cgcgtgaattcatcactgaatgcctctgaag 3' Tm 63°C

The following PCR cycling conditions were used: 10µl 10X Pfu buffer, 1µl 10mM dNTPs, 2µl 10µM each oligo, 83µl sterile water 1.5µl Pfu DNA polymerase (Stratagene, La Jolla, CA), 50 ng DNA (pPDMRa12 WT-1 No His). The reaction was denatured initially at 96°C for 2 minutes, followed by 40 cycles of 96°C for 20 seconds, 62°C for 15 seconds, and 72°C for 1 minute and 40 seconds. This was followed by a final extension of 72°C for 4 minutes. The PCR product was digested with NdeI and

EcoRI and cloned into pPDM His (a modified pET28 vector) that had been digested with NdeI and EcoRI. The construct was confirmed through sequence analysis and then transformed into BLR (DE3) pLys S and HMS 174 (DE3) pLys S cells. This construct – pPDM WT-1 E was then digested with NcoI and XbaI and used as the vector backbone for the NcoI and XbaI insert from pPDM Ra12 WT-1 F (see below). The construct was confirmed through sequence analysis and then transformed into BLR (DE3) pLys S and HMS 174 (DE3) pLys S cells. Protein expression was confirmed by Coomassie stained SDS-PAGE and N-terminal protein sequence analysis.

**Design of Ra12-WT-1-F (a.a. 1-281) with No His tag (SEQ ID NOs:389 (cDNA) and 393 (protein)):**

The Ra12 WT-1 reading frame was PCR amplified with the following primers:

PDM-777 (SEQ ID NO:398) 5' cgataagcatatgacggccgcgtccgataac 3' T<sub>m</sub> 66°C

PDM-779 (SEQ ID NO:399) 5' cgcgtgaattcatcactgaatgcctctgaag 3' T<sub>m</sub> 63°C

The following PCR cycling conditions were used: 10µl 10X Pfu buffer, 1µl 10mM dNTPs, 2µl 10µM each oligo, 83µl sterile water 1.5µl Pfu DNA polymerase (Stratagene, La Jolla, CA), 50 ng DNA (pPDMRa12 WT-1 No His). The reaction was denatured initially at 96°C for 2 minutes, followed by 40 cycles of 96°C for 20 seconds, 58°C for 15 seconds, and 72°C for 3 minutes. This was followed by a final extension of 72°C for 4 minutes. The PCR product was digested with NdeI and cloned into pPDM His that had been digested with NdeI and EcoRI. The sequence was confirmed through sequence analysis and then transformed into BLR (DE3) pLys S and HMS 174 (DE3) pLysS cells. Protein expression was confirmed by Coomassie stained SDS-PAGE and N-terminal protein sequence analysis.

**Design of Ra12-WT-1 with No His tag (SEQ ID NOs:390 (cDNA) and 392 (protein)):**

The Ra12 WT-1 reading frame was PCR amplified with the following primers:

PDM-777 (SEQ ID NO:400) 5' cgataagcatatgacggccgctccgataac 3' Tm  
66°C

PDM-778 (SEQ ID NO:401) 5' gtctgcagcggccgctcaaagcgccagc 3' Tm  
70°C

5 The following PCR cycling conditions were used: 10µl 10X Pfu buffer,  
1µl 10mM dNTPs, 2µl 10µM each oligo, 83µl sterile water 1.5µl Pfu DNA polymerase  
(Stratagene, La Jolla, CA), 50 ng DNA (pPDMRa12 WT-1 No His). The reaction was  
denatured initially at 96°C for 2 minutes, followed by 40 cycles of 96°C for 20 seconds,  
68°C for 15 seconds, and 72°C for 2 minutes and 30 seconds. This was followed by a  
10 final extension of 72°C for 4 minutes. The PCR product was digested with NotI and  
NdeI and cloned into pPDM His that had been digested with NdeI and NotI. The  
sequence was confirmed through sequence analysis and then transformed into BLR  
(DE3) pLys S and HMS 174 (DE3) pLysS cells. Protein expression was confirmed by  
Coomassie stained SDS-PAGE and N-terminal protein sequence analysis.

15 **Design of WT-1 C (a.a. 69-430) in *E. coli* without His tag (SEQ ID  
NOs:387 (cDNA) and 394 (protein)):**

The WT-1 C reading frame was PCR amplified with the following  
primers:

20 PDM-780 (SEQ ID NO:402) 5' gacgaaagcatatgcactcctcatcaaac 3' Tm  
60°C

PDM-778 (SEQ ID NO:403) 5' gtctgcagcggccgctcaaagcgccagc 3' Tm  
70°C

25 The following PCR cycling conditions were used: 10µl 10X Pfu buffer,  
1µl 10mM dNTPs, 2µl 10µM each oligo, 83µl sterile water 1.5µl Pfu DNA polymerase  
(Stratagene, La Jolla, CA), 50 ng DNA (pPDMRa12 WT-1 No His). The reaction was  
denatured initially at 96°C for 2 minutes, followed by 40 cycles of 96°C for 20 seconds,  
62°C for 15 seconds, and 72°C for 2 minutes. This was followed by a final extension of  
72°C for 4 minutes. The PCR product was digested with NdeI and cloned into pPDM  
His that had been digested with NdeI and Eco72I. The sequence was confirmed through  
30 sequence analysis and then transformed into BLR (DE3) pLys S and HMS 174 (DE3)

pLys S cells. Protein expression was confirmed by Coomassie stained SDS-PAGE and N-terminal protein sequence analysis.

#### EXAMPLE 24

##### 5 GENERATION OF WT1-SPECIFIC CD8<sup>+</sup> T CELL CLONES USING WHOLE GENE PRIMING AND IDENTIFICATION OF AN HLA-A2-RESTRICTED WT1 EPIOTOPE

In this example, Adeno and Vaccinia virus delivery vehicles were used to generate WT1-specific T cell lines. A T cell clone from the line was shown to be  
10 specific for WT1 and further, the epitope recognized by this clone was identified.

Dendritic cells (DC) were differentiated from monocyte cultures derived from PBMC of normal donors by growth for 4-6 days in RPMI medium containing 10% human serum, 50 ng/ml GM-CSF and 30 ng/ml IL-4. Following culture, DC were infected 16 hours with recombinant WT1-expressing vaccinia virus at a multiplicity of  
15 infection (MOI) of 5 or for 2-3 days with recombinant WT1-expressing adeno virus at an MOI of 3-10. Vaccinia virus was inactivated by U.V. irradiation. CD8<sup>+</sup> T-cells were isolated by negative depletion using antibodies to CD4, CD14, CD16, CD19 and CD56<sup>+</sup> cells, followed by magnetic beads specific for the Fc portion of these Abs.

Priming cultures were initiated in 96-well plates. Cultures were  
20 restimulated every 7-14 days using autologous dendritic cells infected with adeno or vaccinia virus engineered to express WT1. Following 4-5 stimulation cycles, CD8<sup>+</sup> T cell lines could be identified that specifically produced interferon- $\gamma$  (IFN- $\gamma$ ) when stimulated with autologous-WT1 expressing dendritic cells or fibroblasts. These lines were cloned and demonstrated to specifically recognize WT1 transduced autologous  
25 fibroblasts but not control transduced fibroblasts by Elispot assays.

To further analyze HLA restriction of these WT1 specific CD8<sup>+</sup> T-cell clones, fibroblasts derived from an additional donor (D475), sharing only the HLA-A2 allele with the donor (D349) from which the T-cell clone was established, were transduced with WT1. ELISPOT analysis demonstrated recognition of these D475  
30 target cells by the T-cell clone. To further demonstrate HLA A2 restriction and demonstrate that this epitope is expressed by tumor cells "naturally" overexpressing WT1



(as part of their malignant transformation), the leukemia cell line K562 was tested. K562 was transduced with the HLA A2 molecule, and HLA-A2 negative K562 cells were used as controls for nonspecific IFN- $\gamma$  release. ELISPOT analysis demonstrated that the T cells recognized the A2 positive K562 cell line, but not the A2 negative K562 cells. Further proof of specificity and HLA-A2 restriction of the recognition was documented by HLA-A2 antibody blocking experiments.

To further define the WT1 epitope, 4 truncated WT1 retroviral constructs were generated. Donor 475 fibroblasts were then transduced with these constructs. ELISPOT assays demonstrated recognition of D475 fibroblasts transduced with the WT1 Tr1 construct (aa2-aa92), thus demonstrating that the WT1 epitope is localized within the first 91 N-terminal amino acids of the WT1 protein. To fine map the epitope, 15mer peptides of the WT1 protein, overlapping by 11 amino acids, were synthesized. The WT1 specific T-cell clone recognized two overlapping 15mer peptides, peptide 9 (QWAPVLDFAPPGASA) (SEQ ID NO: 412) and peptide 10 (VLDFAPPGASAYGSL) (SEQ ID NO: 413). To further characterize the minimal epitope recognized, shared 9mer and 10mer peptides of the 15mers (5 total) were used to analyse the specificity of the clone. The clone specifically recognized the 9mer, VLDFAPPGA (SEQ ID NO:241), and the 10mer, VLDFAPPGAS (SEQ ID NO:411).

20

## EXAMPLE 25

CLONING AND SEQUENCING OF TCR ALPHA AND BETA CHAINS DERIVED FROM A CD8 T CELL SPECIFIC FOR WT1

T cell receptor (TCR) alpha and beta chains from CD8<sup>+</sup> T cell clones specific for WT1 are cloned. Sequence analysis is carried to demonstrate the family origin of the the alpha and beta chains of the TCR. Additionally, unique diversity and joining segments (contributing to the specificity of the response) are identified.

Total mRNA from  $2 \times 10^6$  cells from a WT1 specific CD8<sup>+</sup> T cell clone is isolated using Trizol reagent and cDNA is synthesized using Ready-to-go kits (Pharmacia). To determine V $\alpha$  and V $\beta$  sequences in a clone, a panel of V $\alpha$  and V $\beta$  subtype specific primers are synthesized (based on primer sequences generated by

Clontech, Palo Alto, CA) and used in RT-PCR reactions with cDNA generated from each clone. The RT-PCR reactions demonstrate which V $\beta$  and V $\alpha$  sequence is expressed by each clone.

To clone the full-length TCR alpha and beta chains from a clone, primers  
5 are designed that span the initiator and terminator-coding TCR nucleotides. Standard  
35 cycle RT-PCR reactions are established using cDNA synthesized from the CTL  
clone and the above primers using the proofreading thermostable polymerase PWO  
(Roche, Basel, Switzerland). The resultant specific bands (~850 bp for alpha and ~950  
for beta) are ligated into the PCR blunt vector (Invitrogen, Carlsbad, CA) and  
10 transformed into *E.coli*. *E.coli* transformed with plasmids containing full-length alpha  
and beta chains are identified, and large scale preparations of the corresponding  
plasmids are generated. Plasmids containing full-length TCR alpha and beta chains are  
then sequenced using standard methods. The diversity-joining (DJ) region that  
contributes to the specificity of the TCR is thus determined.

15

From the foregoing it will be appreciated that, although specific  
embodiments of the invention have been described herein for purposes of illustration,  
various modifications may be made without deviating from the spirit and scope of the  
20 invention. Accordingly, the invention is not limited except as by the appended claims.

## CLAIMS

What is Claimed:

1. An isolated polynucleotide comprising a sequence selected from the group consisting of:
  - (a) sequences provided in SEQ ID NOs:327-331, 337-341, and 377-390;
  - (b) complements of the sequences provided in SEQ ID NOs:327-331, 337-341, and 377-390;
  - (c) sequences consisting of at least 20 contiguous residues of a sequence provided in SEQ ID NOs:327-331, 337-341, and 377-390;
  - (d) sequences that hybridize to a sequence provided in SEQ ID NOs:327-331, 337-341, and 377-390, under moderately stringent conditions;
  - (e) sequences having at least 75% identity to a sequence of SEQ ID NOs:327-331, 337-341, and 377-390;
  - (f) sequences having at least 90% identity to a sequence of SEQ ID NOs:327-331, 337-341, and 377-390; and
  - (g) degenerate variants of a sequence provided in SEQ ID NOs:327-331, 337-341, and 377-390.
2. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:
  - (a) sequences encoded by a polynucleotide of claim 1; and
  - (b) sequences having at least 70% identity to a sequence encoded by a polynucleotide of claim 1; and
  - (c) sequences having at least 90% identity to a sequence encoded by a polynucleotide of claim 1;
  - (d) sequences set forth in SEQ ID NOs:241, 332-336, 342-346, 391-395, and 404-413;

- (e) sequences having at least 70% identity to a sequence set forth in SEQ ID NOs:241, 332-336, 342-346, 391-395, and 404-413; and
  - (f) sequences having at least 90% identity to a sequence set forth in SEQ ID NOs:241, 332-336, 342-346, 391-395, and 404-413;
3. An expression vector comprising a polynucleotide of claim 1 operably linked to an expression control sequence.
4. A host cell transformed or transfected with an expression vector according to claim 3.
5. An isolated antibody, or antigen-binding fragment thereof, that specifically binds to a polypeptide of claim 2.
6. A method for detecting the presence of a cancer in a patient, comprising the steps of:
- (a) obtaining a biological sample from the patient;
  - (b) contacting the biological sample with a binding agent that binds to a polypeptide of claim 2;
  - (c) detecting in the sample an amount of polypeptide that binds to the binding agent; and
  - (d) comparing the amount of polypeptide to a predetermined cut-off value and therefrom determining the presence of a cancer in the patient.
7. A fusion protein comprising at least one polypeptide according to claim 2.
8. An oligonucleotide that hybridizes to a sequence recited in SEQ ID NOs:327-331, 337-341, and 377-390 under moderately stringent conditions.

9. A method for stimulating and/or expanding T cells specific for a tumor protein, comprising contacting T cells with at least one component selected from the group consisting of:

- (a) polypeptides according to claim 2;
- (b) polynucleotides according to claim 1; and
- (c) antigen-presenting cells that express a polynucleotide according to claim 1,

under conditions and for a time sufficient to permit the stimulation and/or expansion of T cells.

10. An isolated T cell population, comprising T cells prepared according to the method of claim 9.

11. A composition comprising a first component selected from the group consisting of physiologically acceptable carriers and immunostimulants, and a second component selected from the group consisting of:

- (a) polypeptides according to claim 2;
- (b) polynucleotides according to claim 1;
- (c) antibodies according to claim 5;
- (d) fusion proteins according to claim 7;
- (e) T cell populations according to claim 10; and
- (f) antigen presenting cells that express a polypeptide according to claim 2.

12. A method for stimulating an immune response in a patient, comprising administering to the patient a composition of claim 11.

13. A method for the treatment of a cancer in a patient, comprising administering to the patient a composition of claim 11.

14. A method for determining the presence of a cancer in a patient, comprising the steps of:

- (a) obtaining a biological sample from the patient;
- (b) contacting the biological sample with an oligonucleotide according to claim 8;
- (c) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide; and
- (d) compare the amount of polynucleotide that hybridizes to the oligonucleotide to a predetermined cut-off value, and therefrom determining the presence of the cancer in the patient.

15. A diagnostic kit comprising at least one oligonucleotide according to claim 8.

16. A diagnostic kit comprising at least one antibody according to claim 5 and a detection reagent, wherein the detection reagent comprises a reporter group.

17. A method for inhibiting the development of a cancer in a patient, comprising the steps of:

- (a) incubating CD4<sup>+</sup> and/or CD8<sup>+</sup> T cells isolated from a patient with at least one component selected from the group consisting of: (i) polypeptides according to claim 2; (ii) polynucleotides according to claim 1; and (iii) antigen presenting cells that express a polypeptide of claim 2, such that T cell proliferate;
  - (b) administering to the patient an effective amount of the proliferated T cells,
- and thereby inhibiting the development of a cancer in the patient.

18. A composition comprising a WT1 polypeptide resuspended in a buffer comprising at least one sugar selected from the group consisting of trehalose,

maltose, sucrose, fructose, and glucose, at a concentration of between about 7 and about 13 %.

19. The composition of claim 18 wherein said concentration is between about 8 and about 12%.

20. The composition of claim 18 wherein said concentration is about 10%.

21. A composition comprising a WT1 polypeptide resuspended in a buffer comprising at least 2 sugars selected from the group consisting of trehalose, maltose, sucrose, fructose, and glucose, at a concentration of between about 7 and about 13 %.

22. The composition of claim 21 wherein said concentration is between about 8 and about 12%.

23. The composition of claim 21 wherein said concentration is about 10%.

24. A composition comprising a WT1 polypeptide resuspended in a buffer comprising at least 3 sugars selected from the group consisting of trehalose, maltose, sucrose, fructose, and glucose, at a concentration of between about 7 and about 13 %.

25. The composition of claim 24 wherein said concentration is between about 8 and about 12%.

26. The composition of claim 24 wherein said concentration is about 10%.

27. A composition comprising a WT1 polypeptide resuspended in a buffer comprising:

- (a) at least one sugar selected from the group consisting of trehalose, maltose, sucrose, fructose, and glucose, at a concentration of between about 7 and about 13 %;
- (b) ethanolamine;
- (c) cysteine; and
- (d) Polysorbate-80.

28. The composition of claim 27 wherein said concentration is between about 8 and about 12%.

29. The composition of claim 27 wherein said concentration is about 10%.

30. A composition according to any one of claims 18-29 wherein the WT1 polypeptide comprises an Ra12-WT1 fusion polypeptide.

31. A composition comprising a WT1 polypeptide and MPL-SE.

32. The composition of claim 31 wherein the WT1 polypeptide comprises an Ra12-WT1 fusion polypeptide.

33. A composition comprising a WT1 polypeptide and Enhanzyn.

34. The composition of claim 33 wherein the WT1 polypeptide comprises an Ra12-WT1 fusion polypeptide.



HU:MGSDVRDLNALLPAVPSLGGGGGCGALPVSGAAQWAPVLDFAAPPASAYGSL  
MO:MGSDVRDLNALLPAVSSLGGGGGCGLPVSGAAQWAPVLDFAAPPASAYGSL

HU:GGPAPPPAPPPPPPPPPPHSFQEPKSWGGAEPEEQCLSAFTVHFSGQFTGTAG  
MO:GGPAPPPAPPPPPPPPPPHSFQEPKSWGGAEPEEQCLSAFTLHFSGQFTGTAG

HU:ACRYGPFQPPPPSQASSGQARMFPNAPYLPSCLESQPAIRNQGYSTVTFDGTPS  
MO:ACRYGPFQPPPPSQASSGQARMFPNAPYLPSCLESQPTIRNQGYSTVTFDGAPS

HU:YGHTPSHHAAQFPNHSFKHEDPMGQQGSLGEQQYSVPPPVYGCHTPTDSCGT  
MO:YGHTPSHHAAQFPNHSFKHEDPMGQQGSLGEQQYSVPPPVYGCHTPTDSCGT

HU:SQALLLRTPYSSDNLYQMTSQLECMTNQMNLGATLKGVAAGSSSSSVKWTE  
MO:SQALLLRTPYSSDNLYQMTSQLECMTNQMNLGATLKGMAAGSSSSSVKWTE

HU:GQSNHSTGYESDNHTTPELCAQYRIHTHGTVFRGIQDVRRVPGVAPTLVRSAS  
MO:GQSNHGIGYESDNHTAPELCAQYRIHTHGTVFRGIQDVRRVSGVAPTLVRSAS

HU:ETSEKRPFMCAYPGCNRYFKLSHLQMHRSRKHTEGKPYQCDFKDCERRFSR  
MO:ETSEKRPFMCAYPGCNRYFKLSHLQMHRSRKHTEGKPYQCDFKDCERRFSR

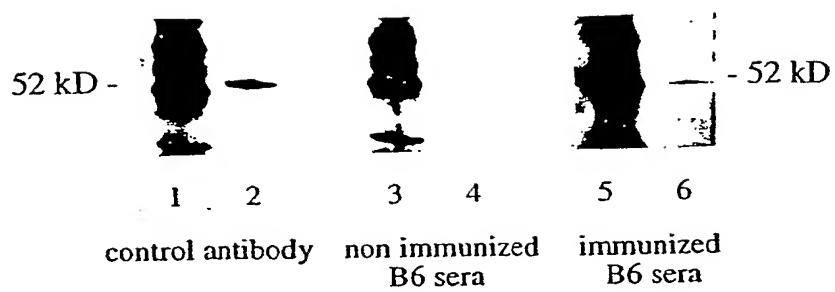
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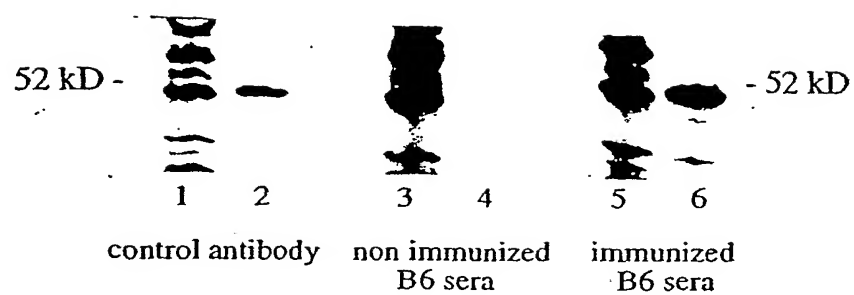
HU:WPSCQKKFARSDELVRHENMHQRNMTKLQAL  
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*FIG. 1*



**FIG. 2**

**FIG. 3**

**FIG. 4**

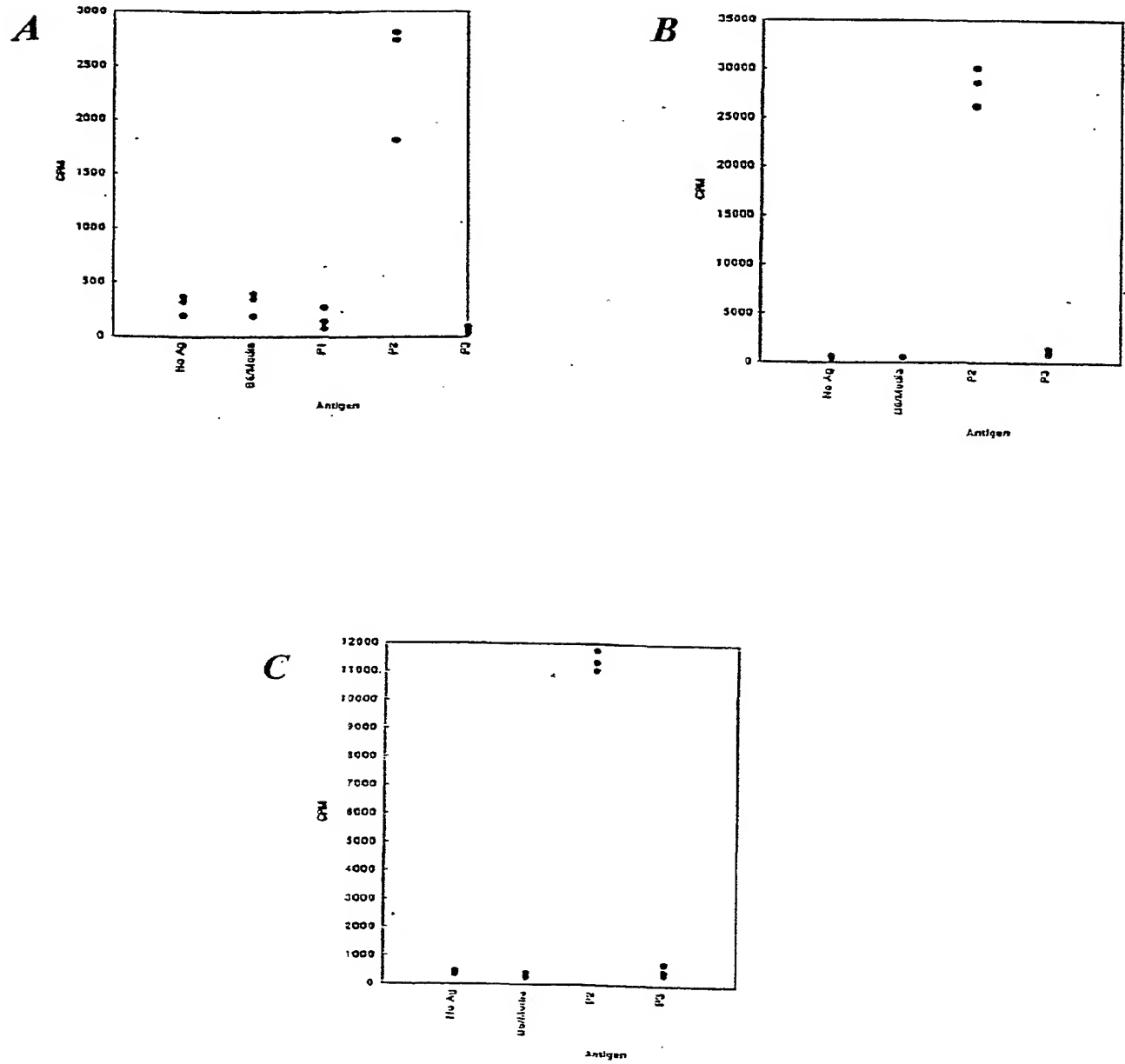
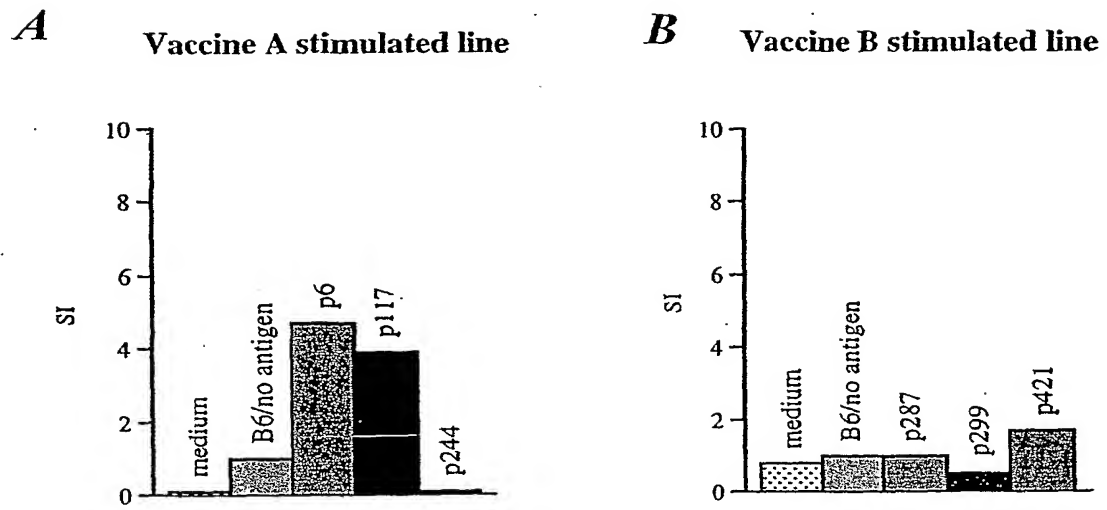
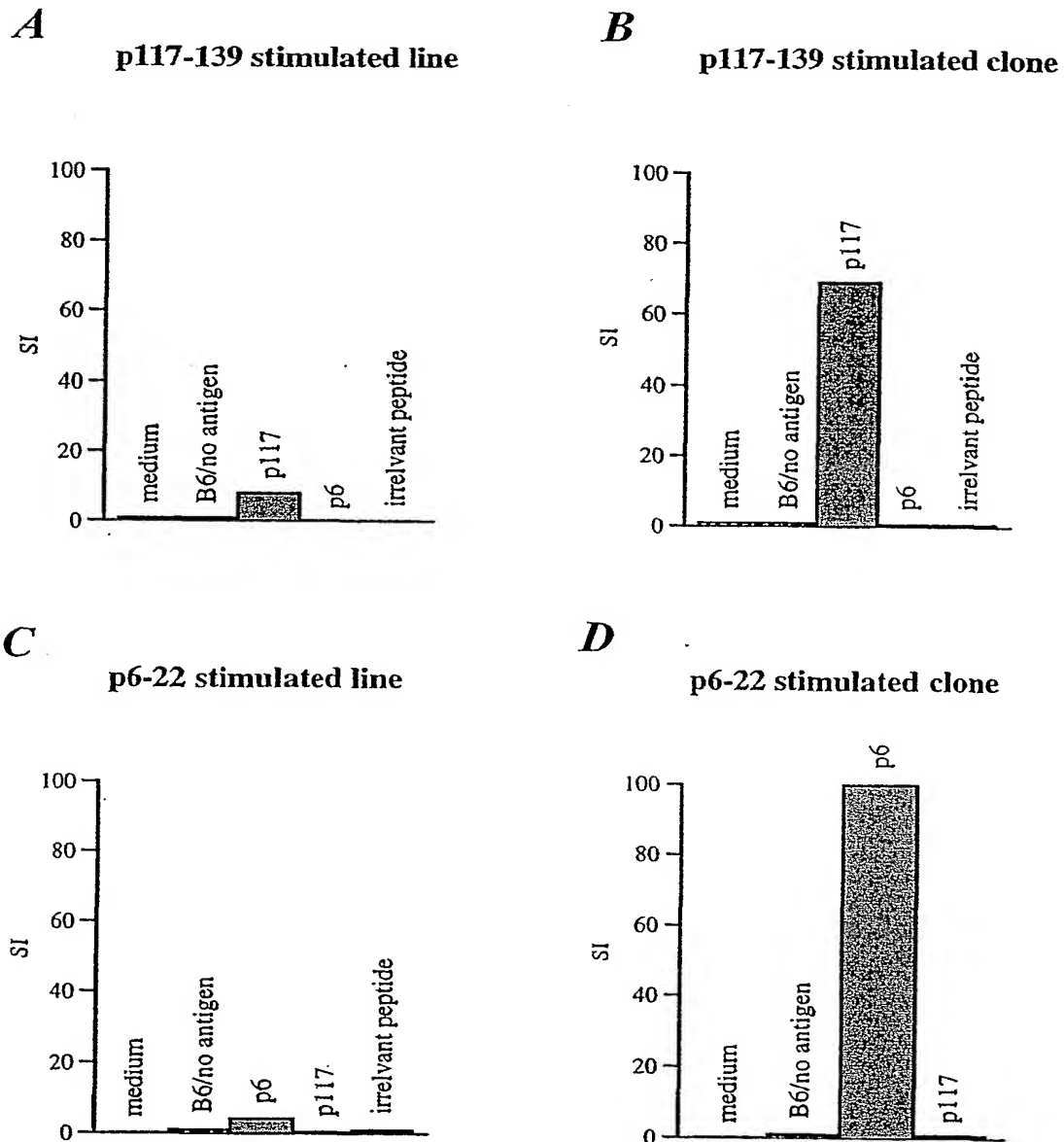


FIG. 5A-5C



**FIG. 6A and 6B**

**FIG. 7A-7D**

```

      5   10   15   20   25   30   35   40   45   50   55   60   65   70   75
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.....RRRR.....
.....
.....

      80   85   90   95  100  105  110  115  120  125  130  135  140  145  150
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.....AAA.....AAAA.....AAA.....AAAAA.....
.....RRRR.....RRRR.....
.....DDDDDDDDDD.....
.....

     155  160  165  170  175  180  185  190  195  200  205  210  215  220  225
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.....RRRR.....
.....DDDDDDDDDDDDDDDD.....
.....

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.....RRRR.....RRRR.....
.....DDDDDD.....
.....

     380  385  390  395  400  405  410  415  420  425  430  435  440  445  450
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.....RRRR.....RRRR.....
.....dddddddddd.....

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FIG. 8A



```

5   10   15   20   25   30   35   40   45   50   55   60   65   70   75
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.....AAAAAAAAAAAAAAAAAAAA.....AAAAA.....AAAAAAAAAAAA.....
.....RRRR.....
.....

80   85   90   95   100  105  110  115  120  125  130  135  140  145  150
PSWGAEPHEEQCLSAFTLHFSGQFTGTAGACRYGPFPGPPPSQASSGQARMFPNAPYLPSCLESQPTIRNQGYS
.....AAAA.....AAA.....AAAAA.....
.....RRRR.....RRRR.....
.....DDDDDDDD.....

155  160  165  170  175  180  185  190  195  200  205  210  215  220  225
TVTFDGAAPSYGHTPSHHAAQFPNHSEKHEDPMGQCGSLGEQQYSVPPVYGCHTPTDSCGTGSQALLLRTPYSSDN
.....AAAA.....AAAAA.....AA
.....RRRR.....
.....DDDDDDDDDDDDDD.....

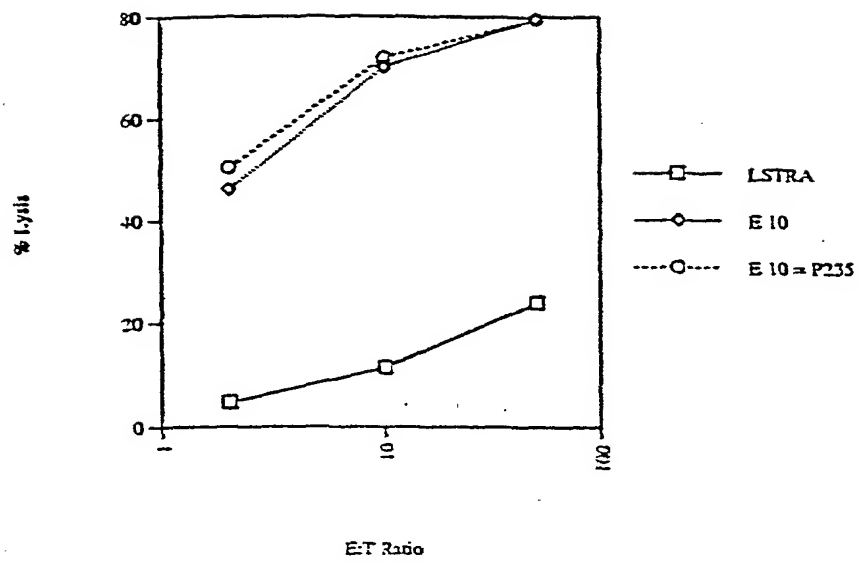
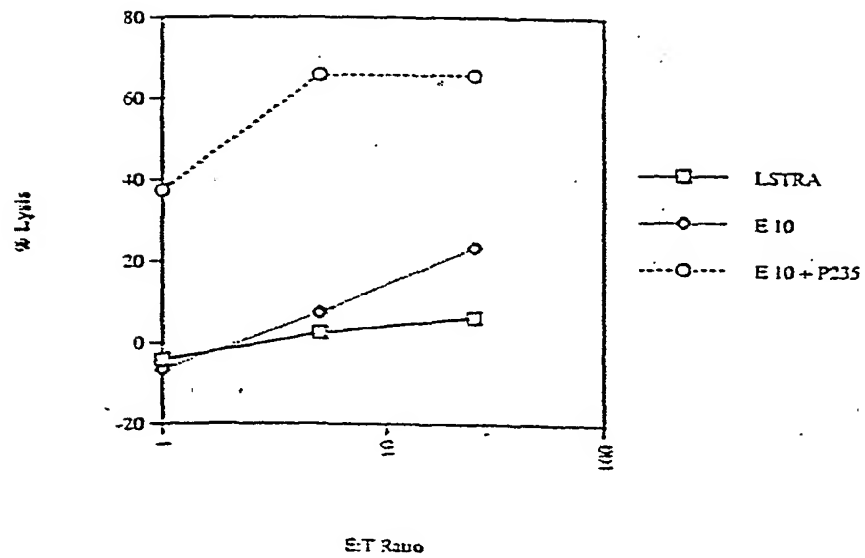
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.....RRRRRRRRRR.....RRRR.....RRRR.....
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.....ddddd.....

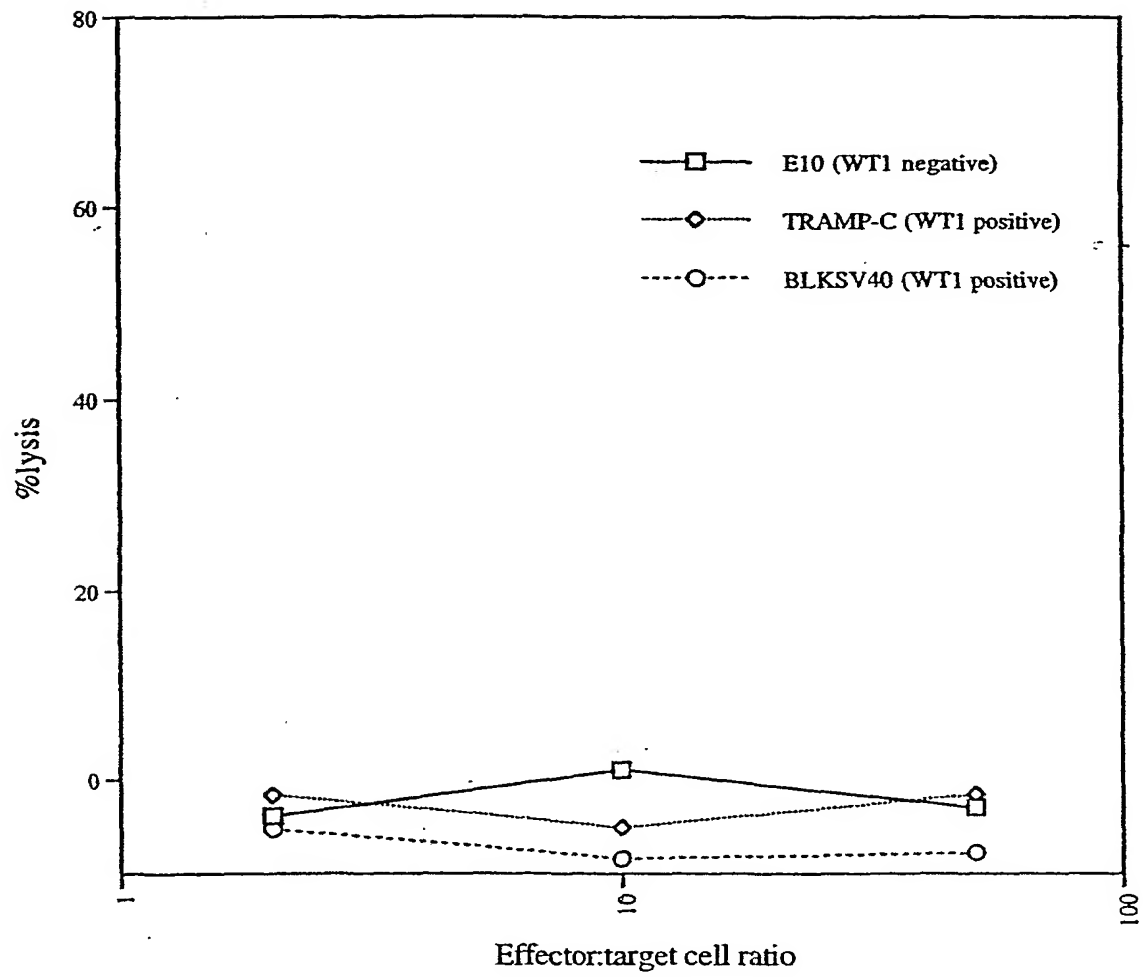
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.....RRRR.....RRRR.....
..DDDDDDDDDDDD.....

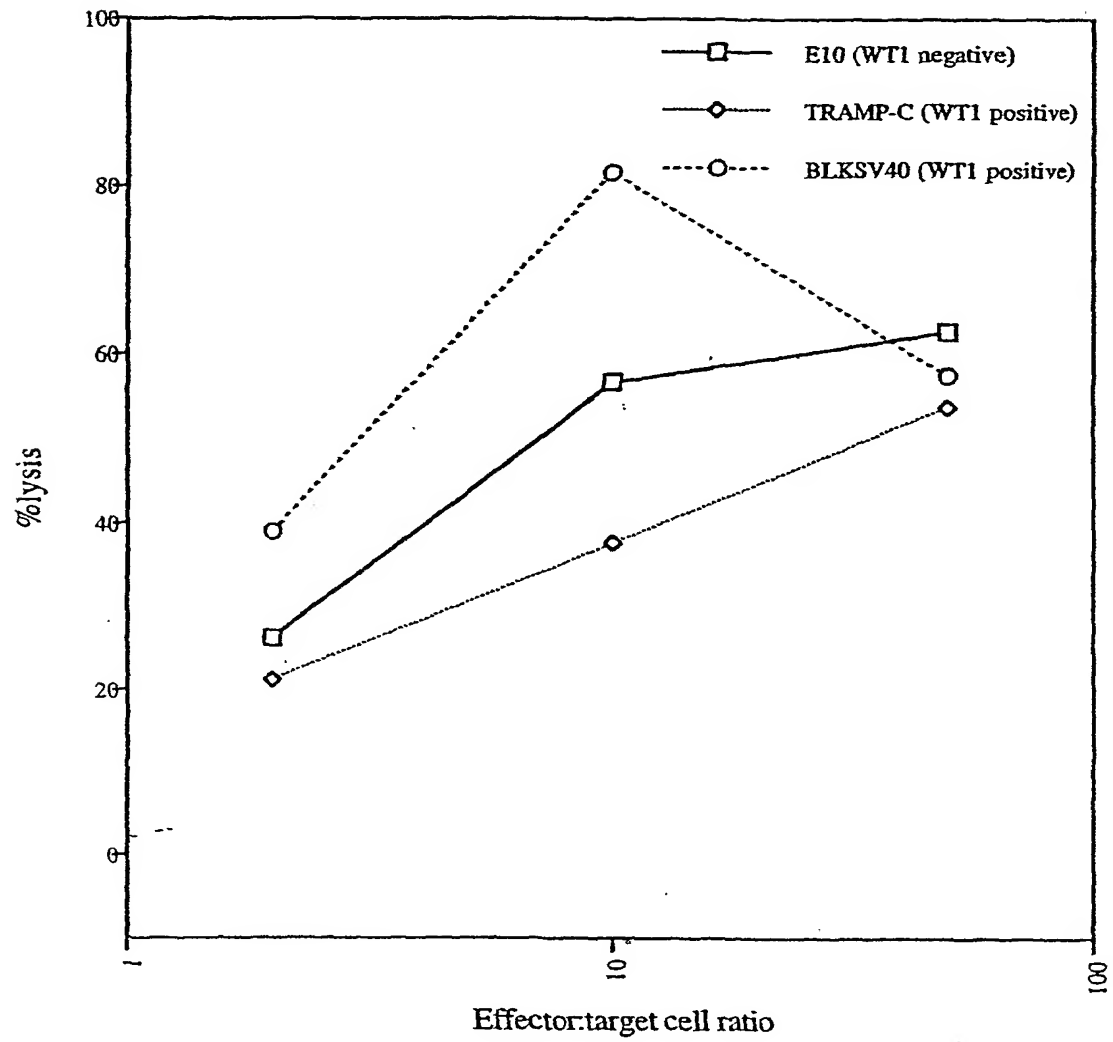
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.....RRRR..RRRR.....
.....dddddddddd.....

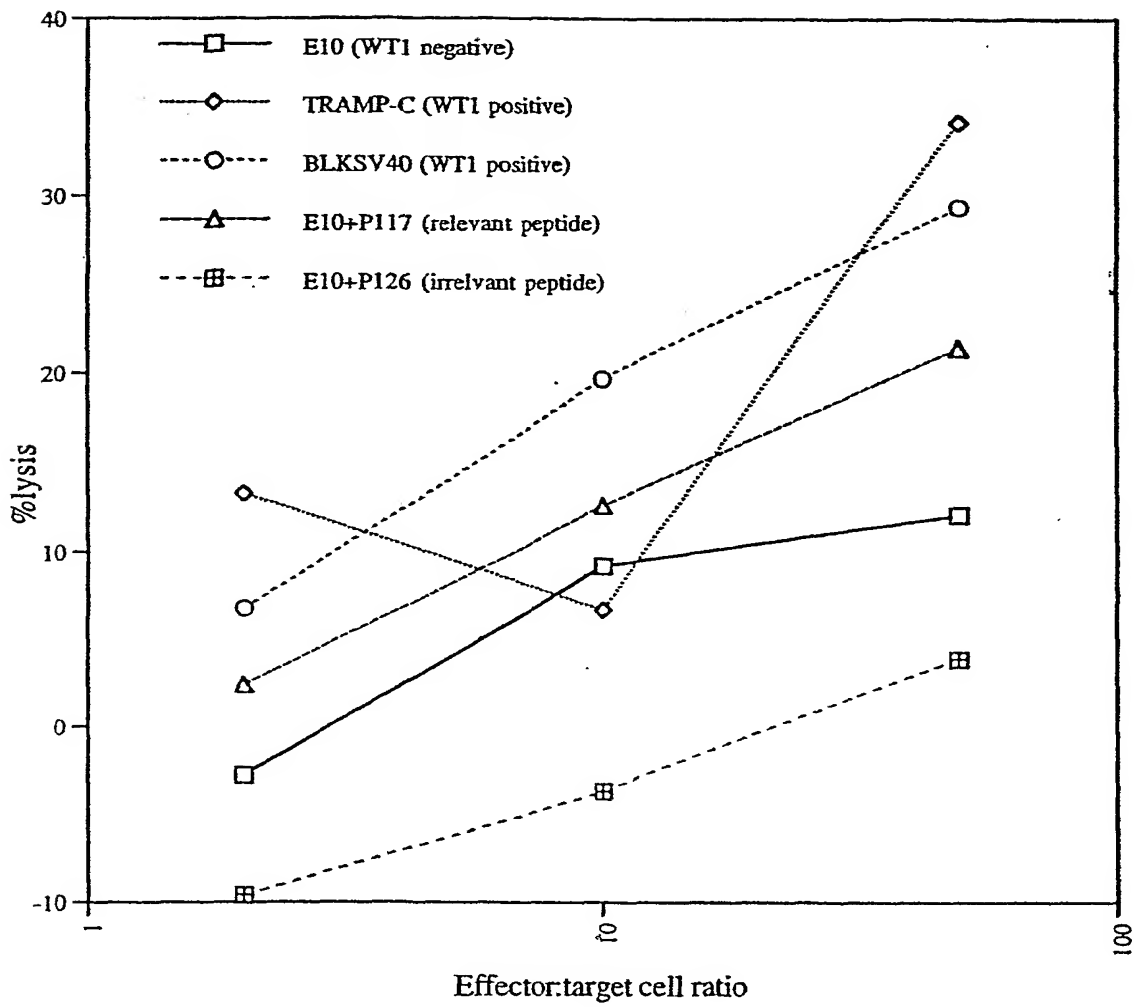
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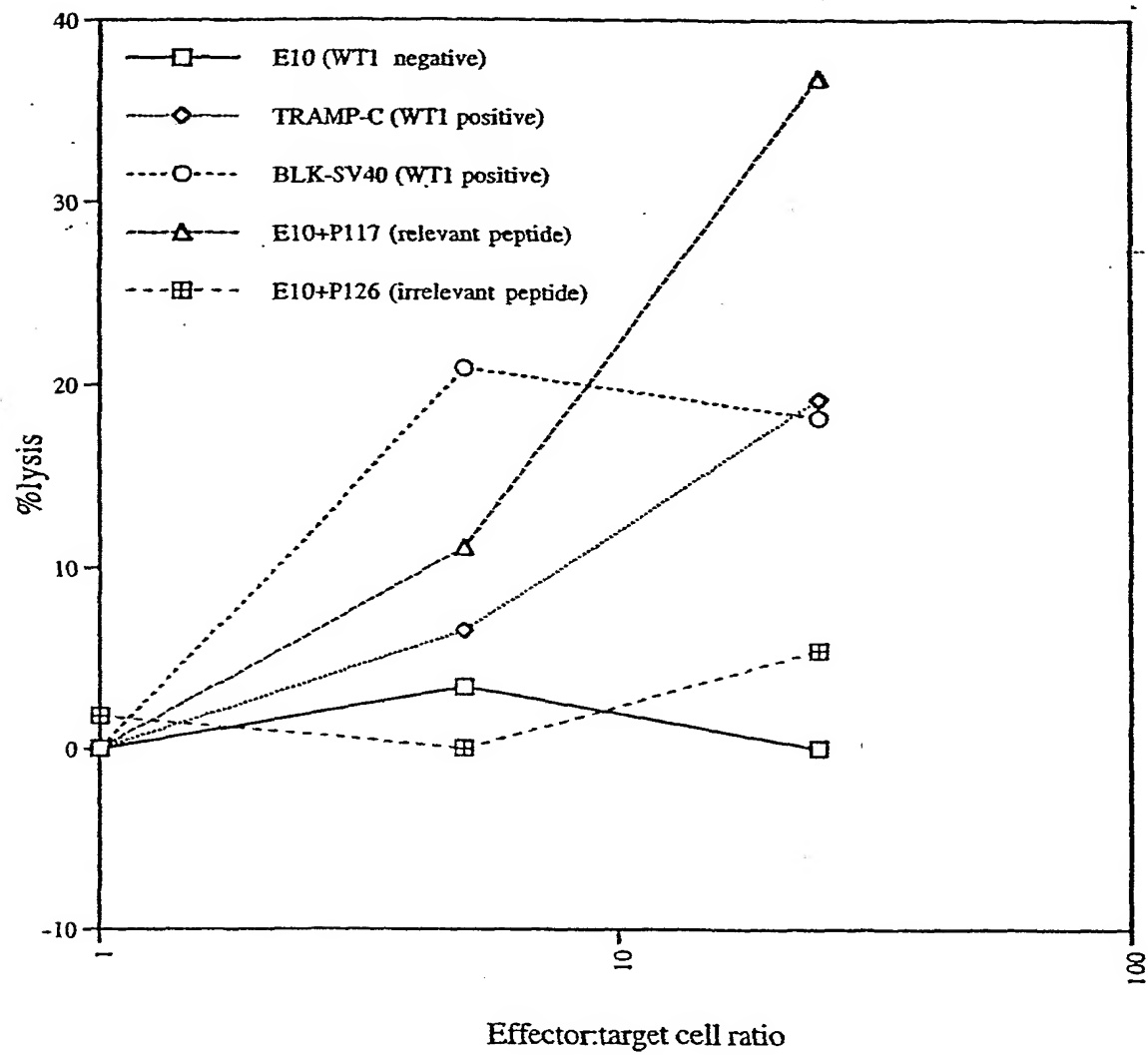
**FIG. 8B**

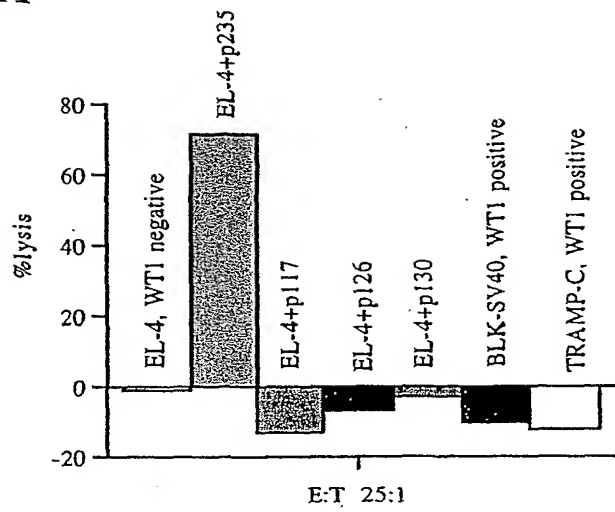
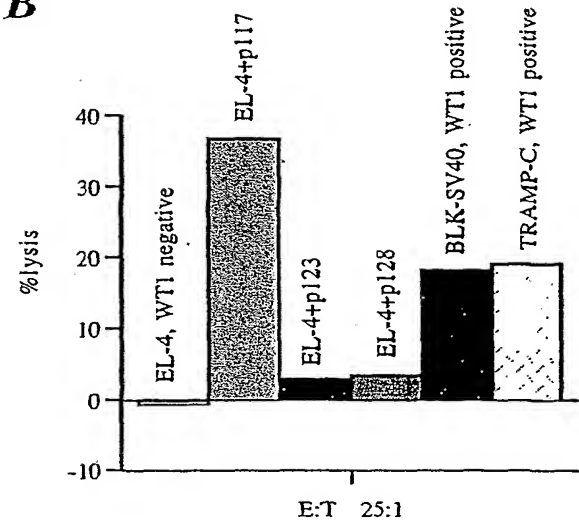
**A****B****FIG. 9A and 9B**

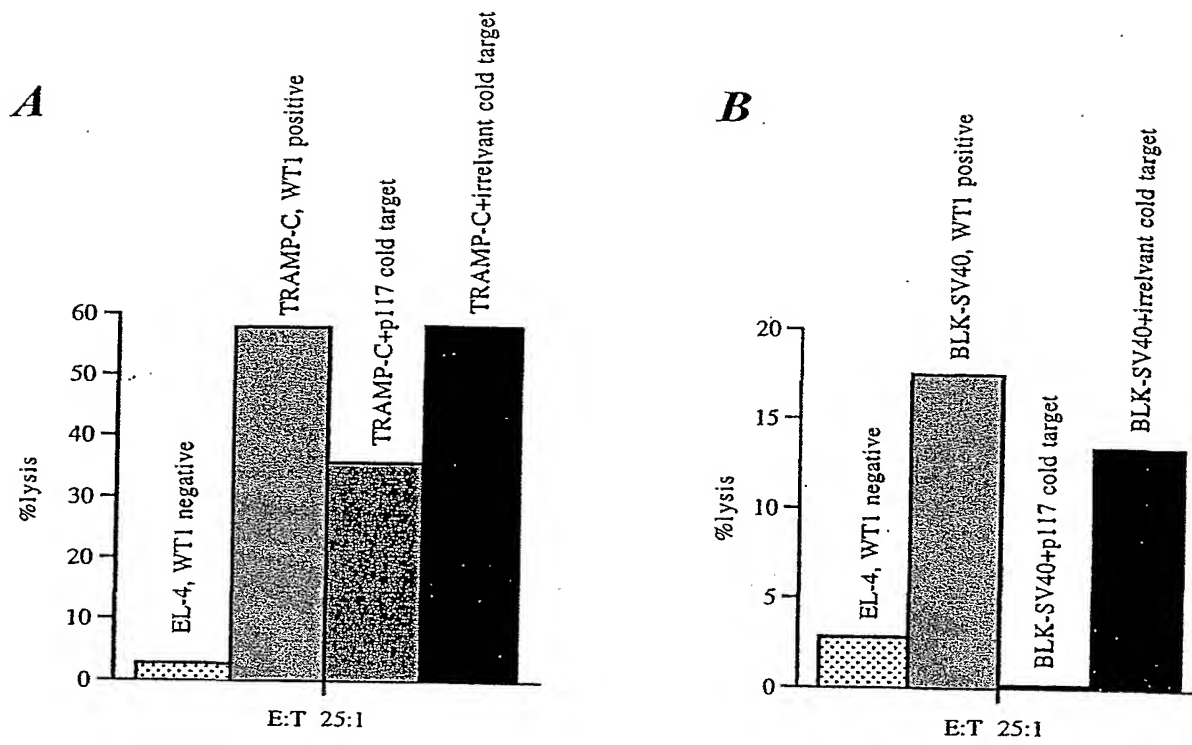
**FIG. 10A**

**FIG. 10B**

*FIG. 10C*

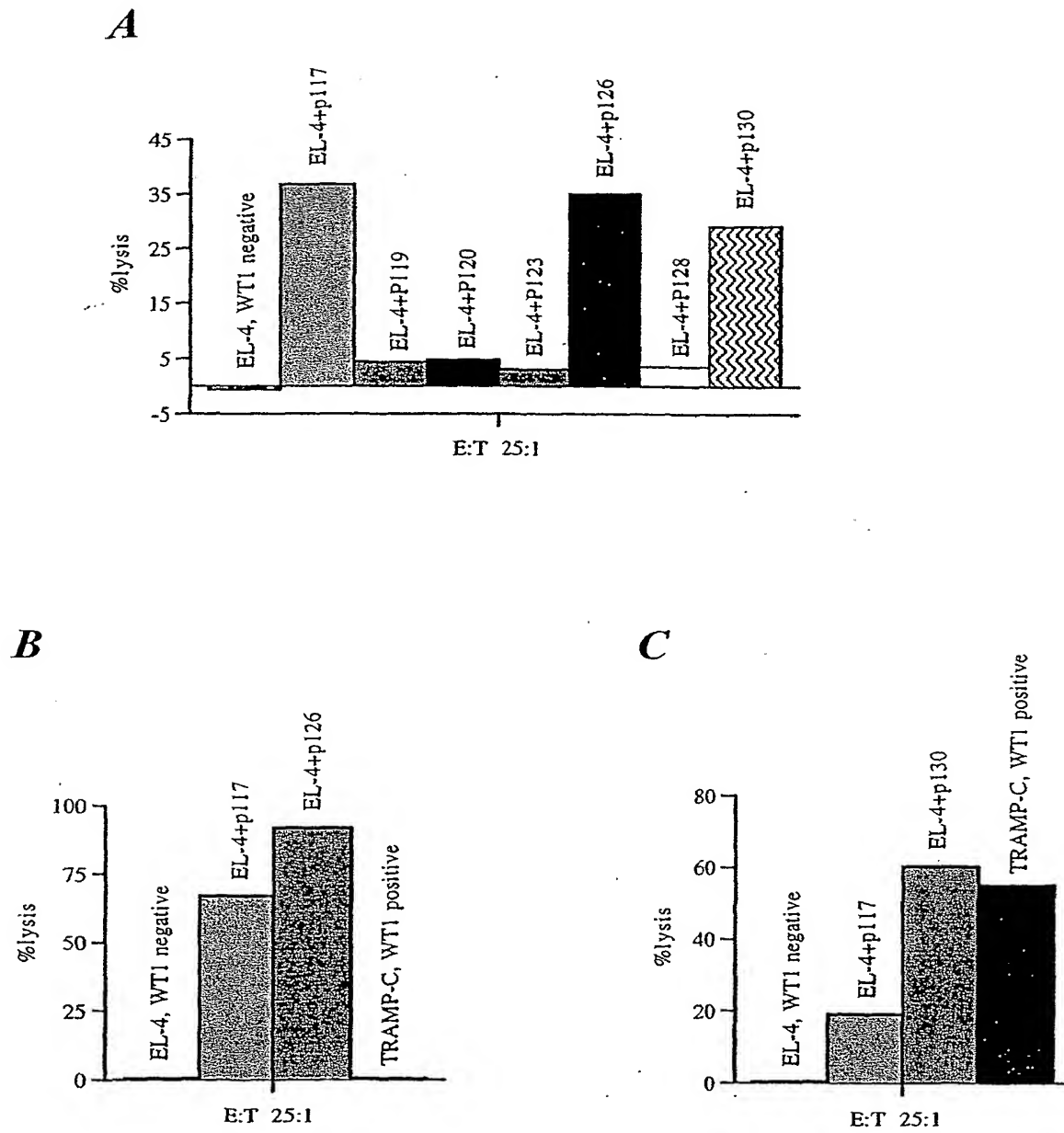
**FIG. 10D**

**A****B****FIG. 11A and 11B**



**FIG. 12A and 12B**



**FIG. 13A-13C**

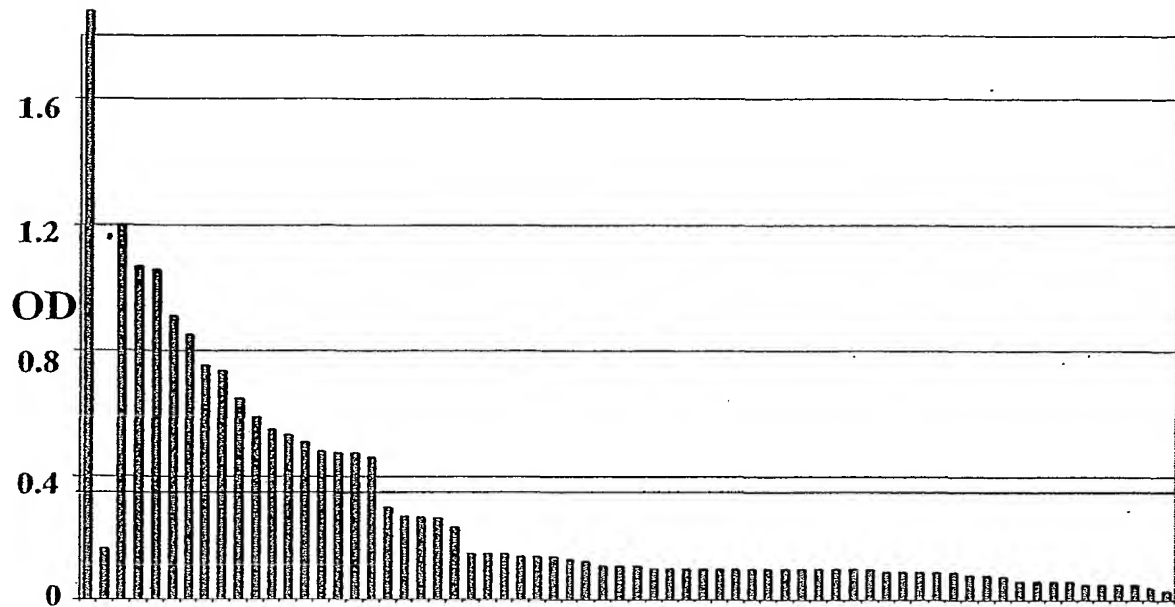


Fig. 14

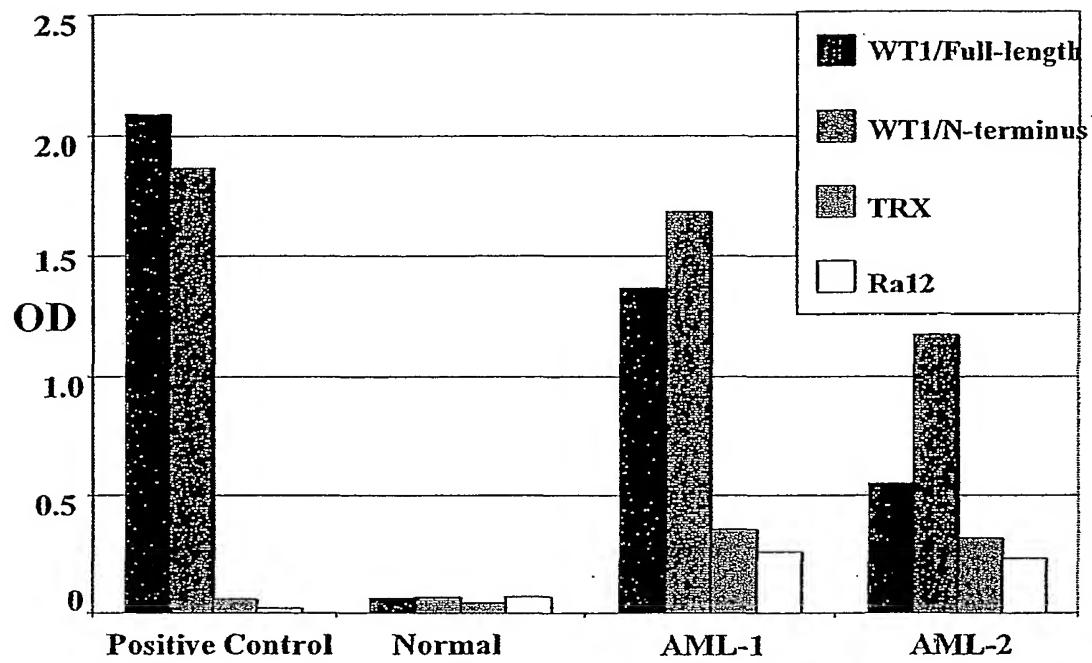


Fig. 15

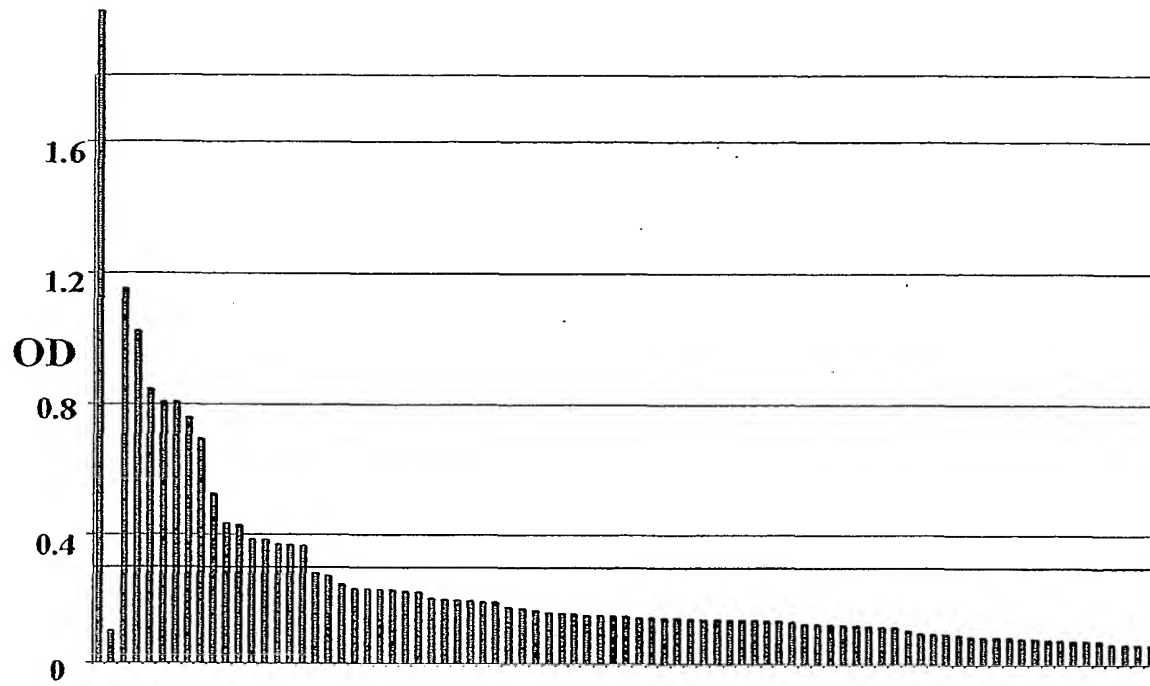


Fig. 16

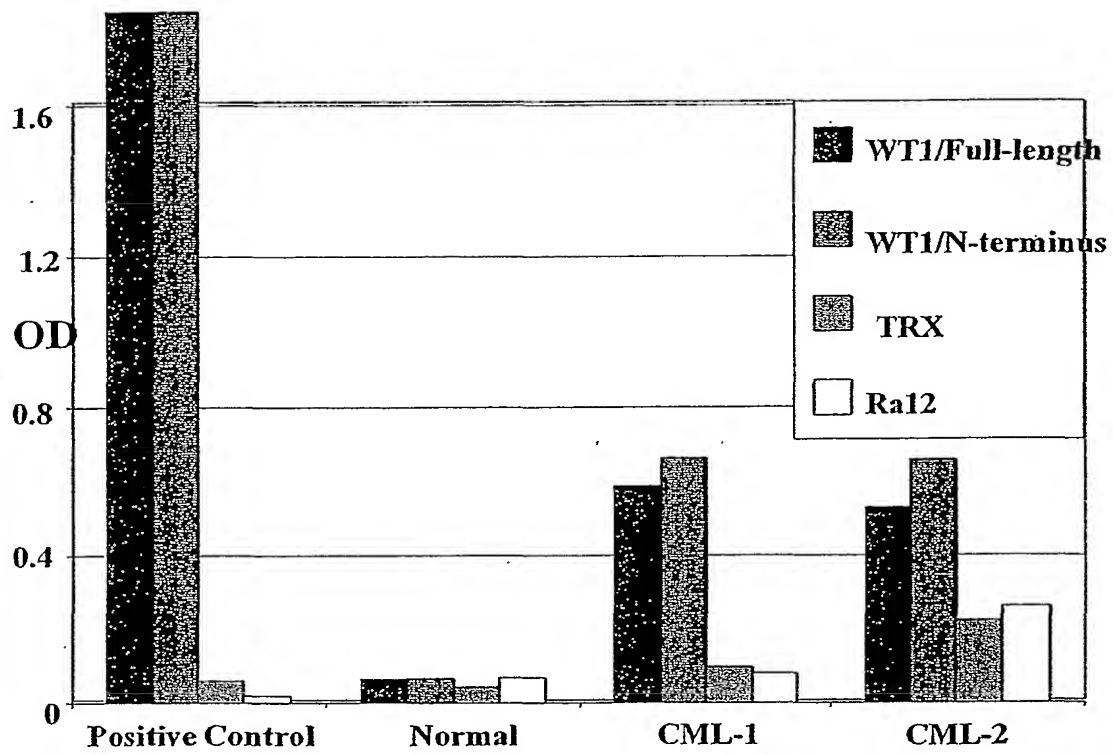


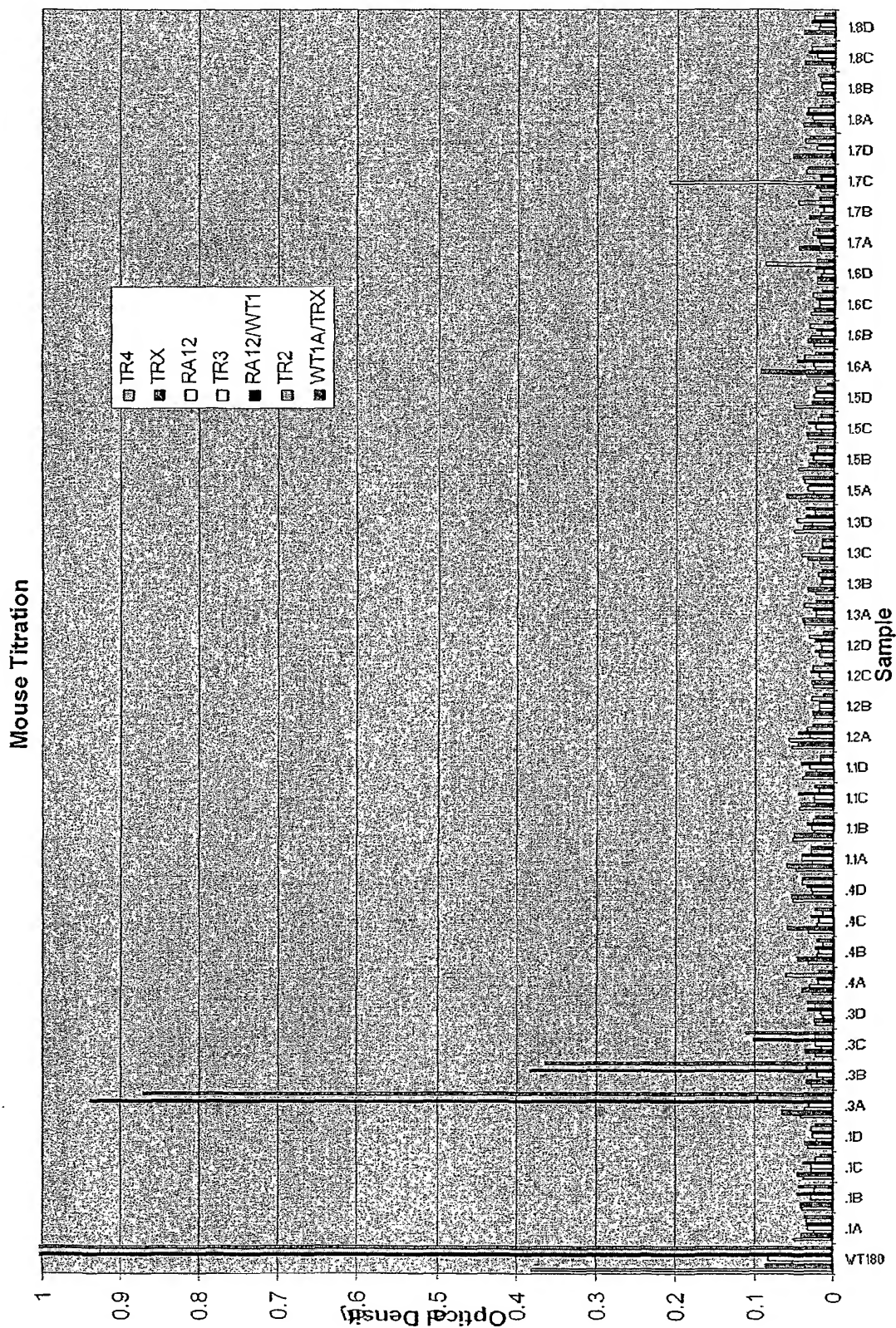
Fig. 17

TABLE 1: Characteristics of Recombinant WT1 Proteins Used for Serological Analysis

<u>Name</u>	<u>Recombinant Protein</u>	<u>WT1 Amino Acid Position</u>	<u>Molecular Weight</u>
WT1/full-length	Ra12-WT1 full length fusion protein	aa 1-449	85kDa
WT1/N-terminus	TRX-WT1 N-terminus fusion protein	aa 1-249	60kDa
WT1/C-terminus	WT1 C-terminus protein	aa 267-449	50kDa

Fig. 18

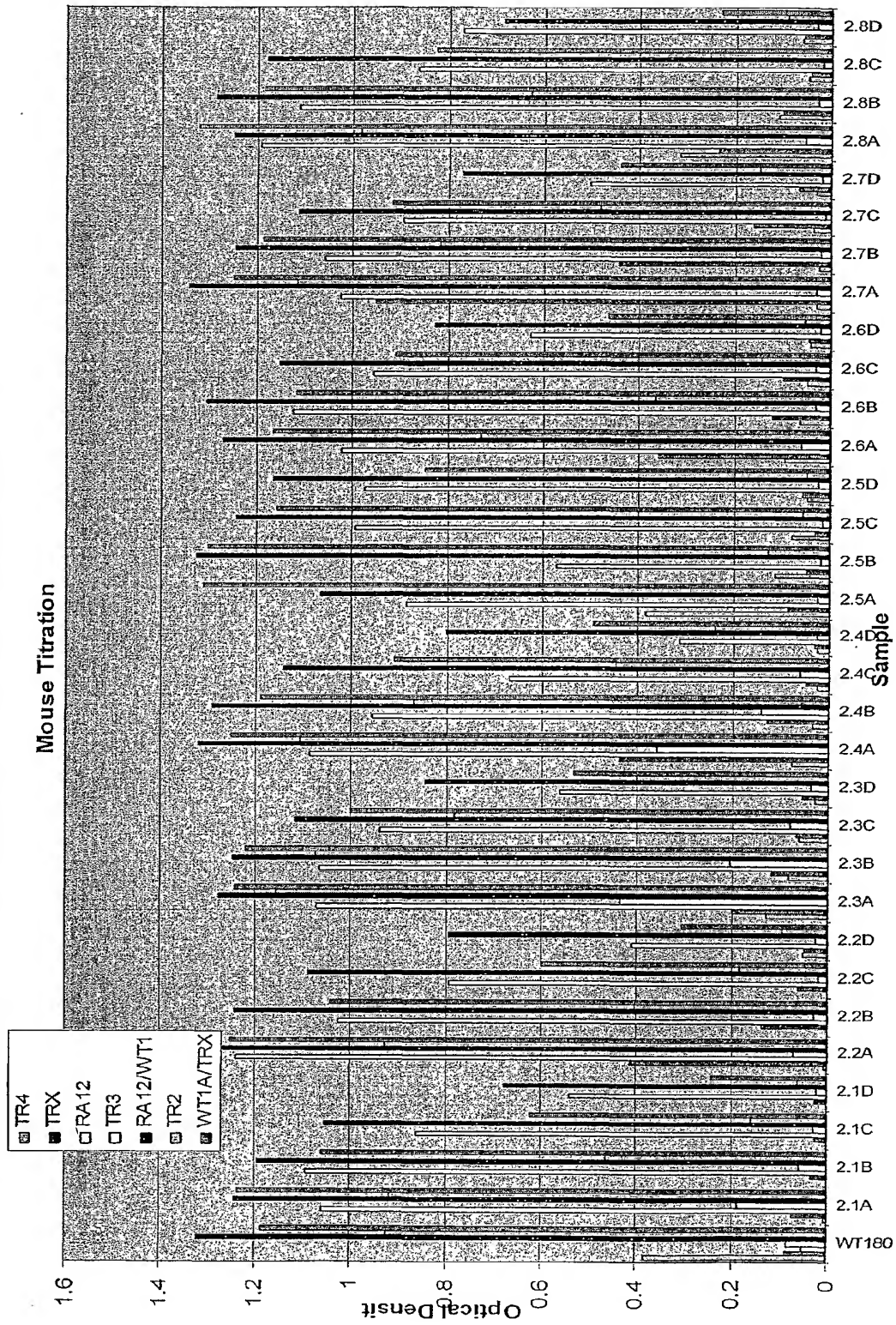
CID000622 Figure 1a Ab responses in group 0 and 1 (controls)



Control groups. A: 1:500 Dilution, B: 1:2000, C: 1: 8000, D: 1:16000

FIG. 19 A

CID000622 Figure 1b. Ab responses in group 2 (25ug Ra12/WT1)

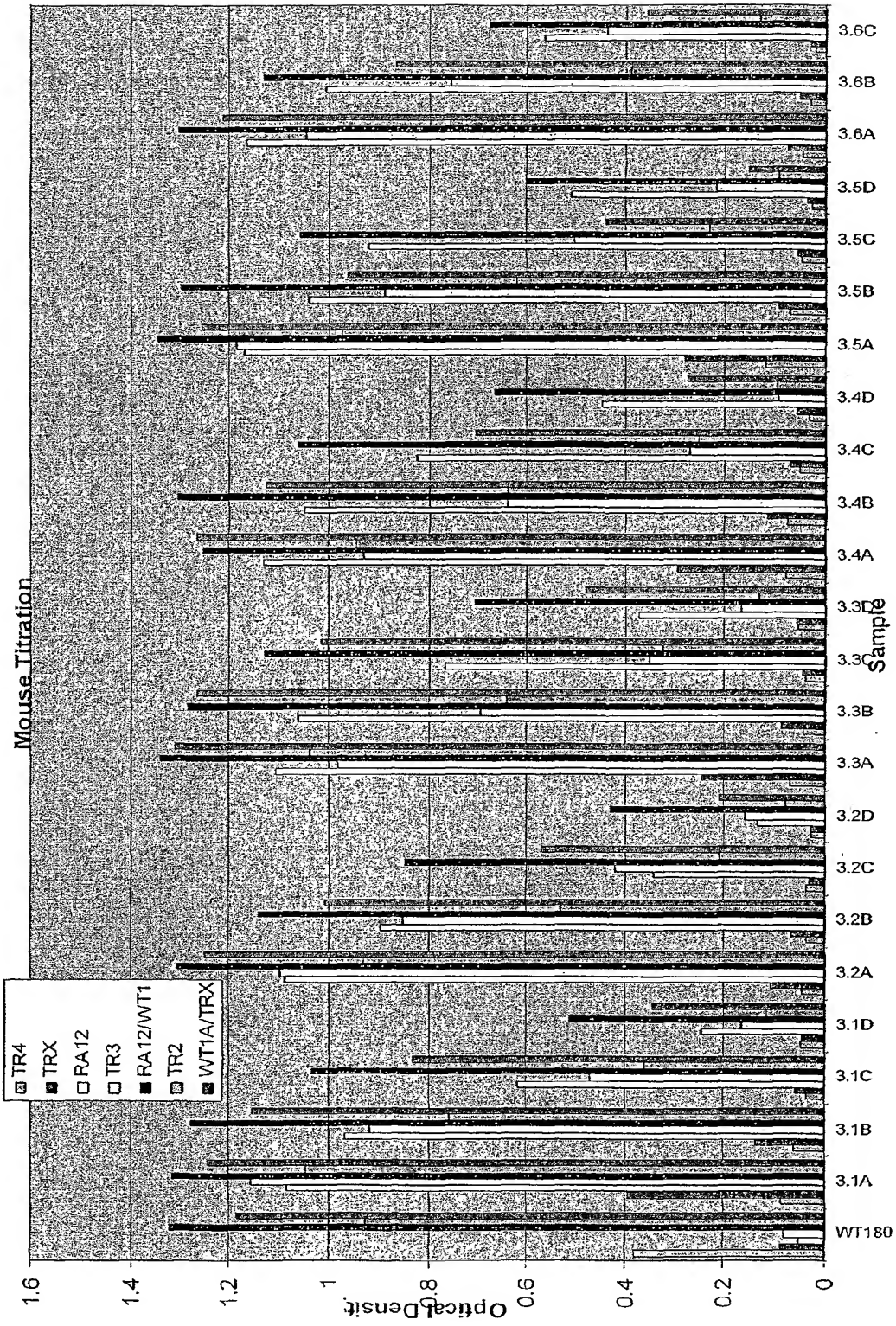


25ug Ra12/WT1+MPL-SE, A: 1:500 Dilution, B: 1:2000, C: 1: 8000, D: 1:16000

FIG. 19B



CID000622 Figure 1c. Ab responses in group 3 (100ug Ra12/WT1)

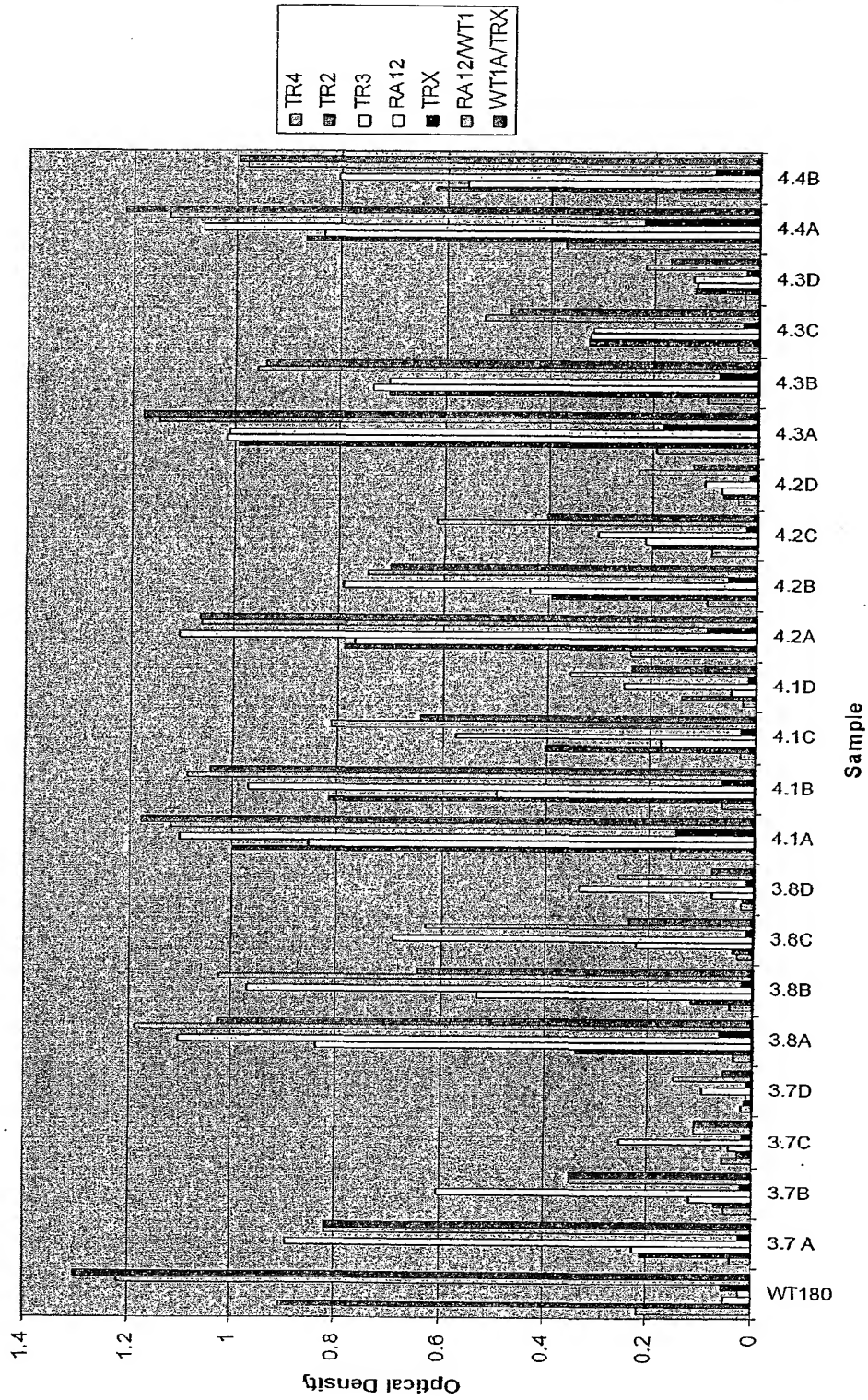


WT1. Dose Titration. Ab responses to WT1. 100ug Ra12-WT1+MPL-SE. A: 1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000

FIG. 19C

CID000622 Figure 1d. Ab responses in groups 3 and 4 (1000ug Ra12/WT1)

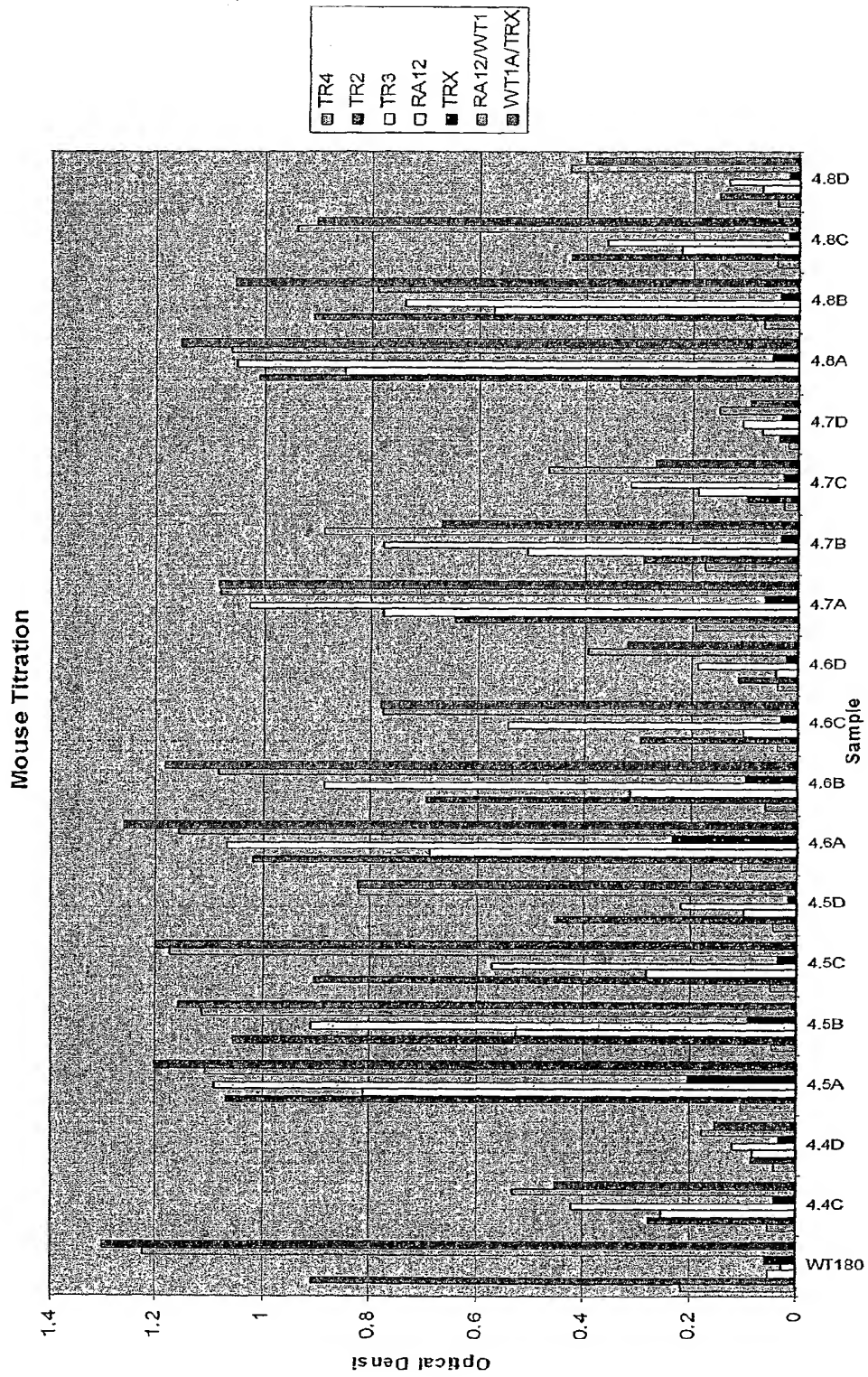
Mouse Titration



WT1. Dose Titration. Ab responses to WT1. 1000ug Ra12-WT1+MPL-SE. A: 1;500 Dilution, B: 1:2000, C: 1: 8000, D: 1:16000

FIG. 19D

Figure 1e. Ab responses in group 4 (1000ug Ra12/WT1)



WT1. Dose Titration. Ab responses to WT1. 1000ug Ra12-WT1+MPL-SE. A: 1;500 Dilution,  
B: 1:2000, C: 1: 8000, D: 1:16000

FIG. 19E

Figure 2a. Proliferative T-cell responses in WT1 protein immunized mice.  
(Ra12WT1 dose titration, 3x in vivo, after 2IVS)

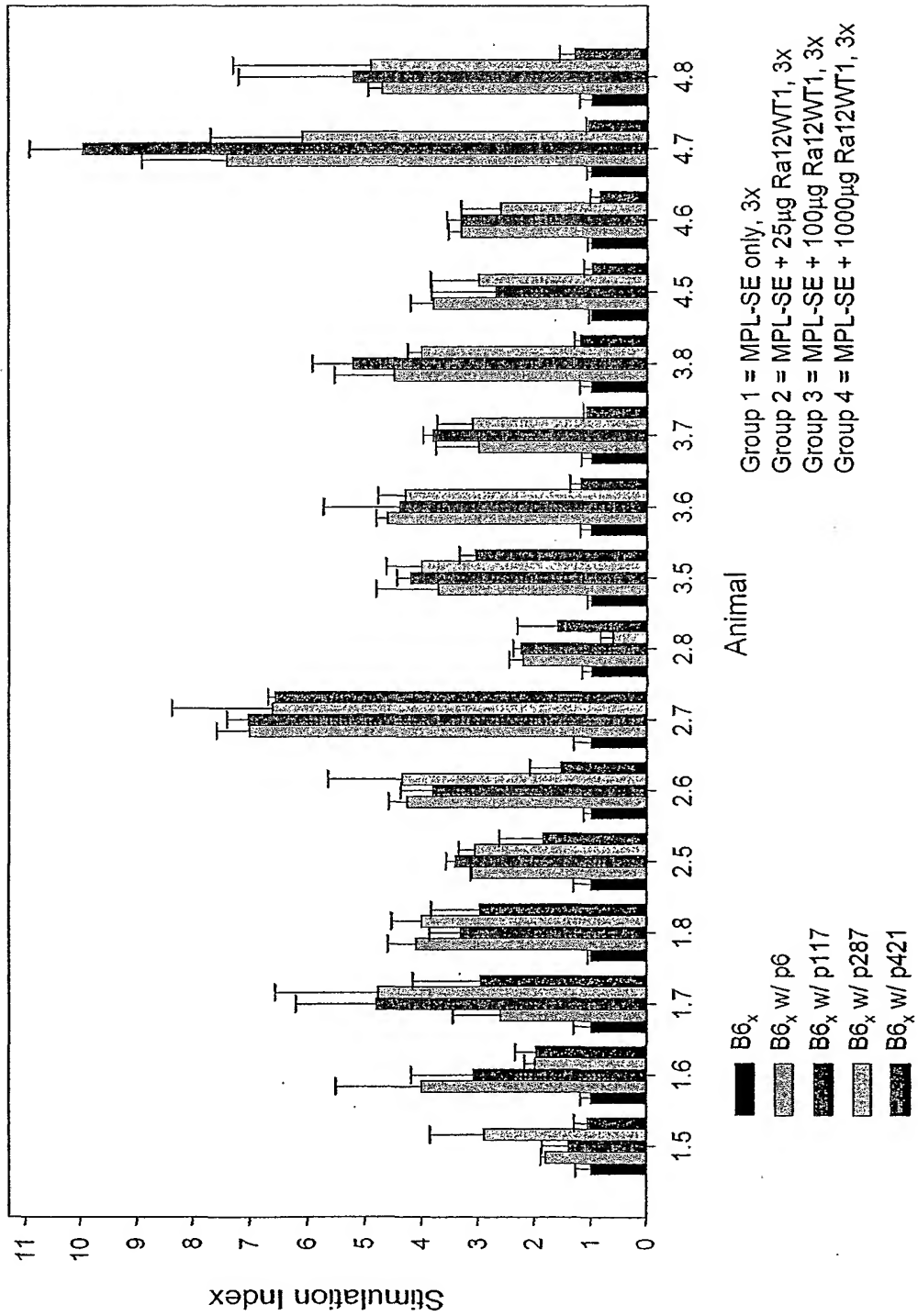


FIG. 20A

Figure 2b. Proliferative T-cell responses in WT1 protein immunized mice (Ra12WT1 dose titration, 6x in vivo, after 2IVS)

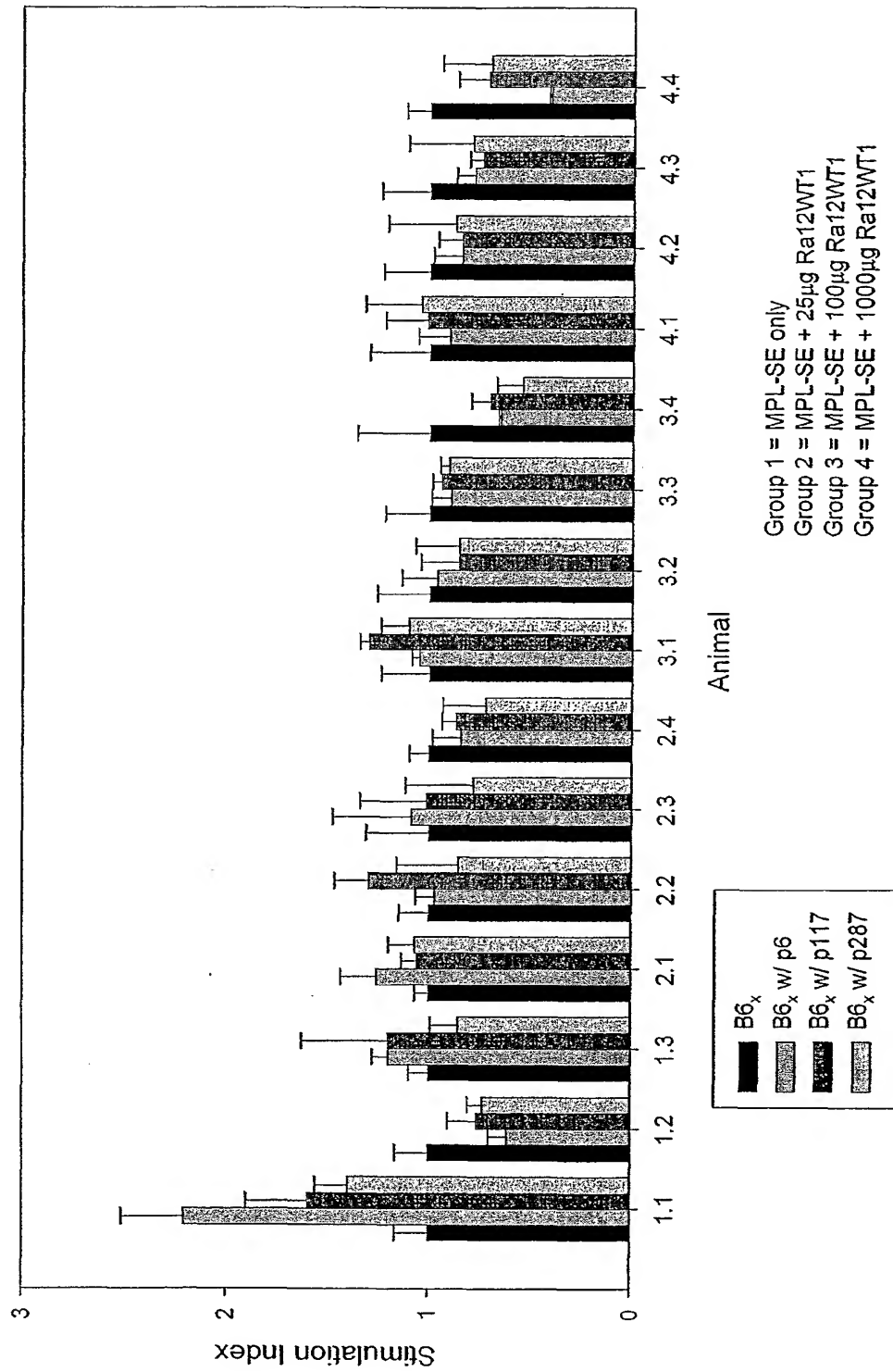


FIG. 20B



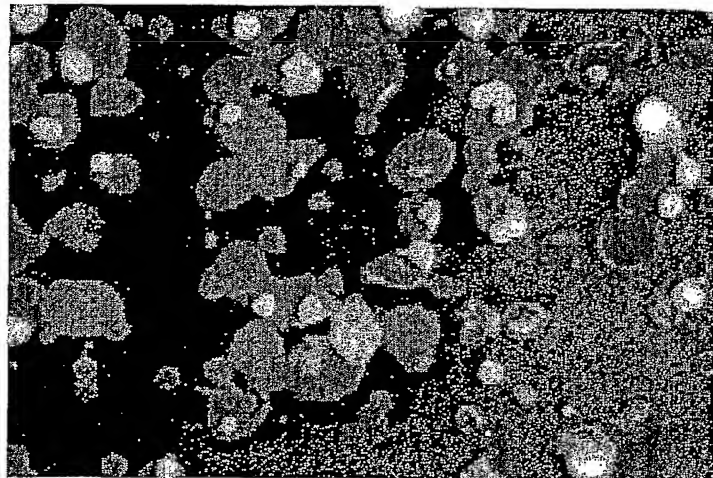
FIG. 21

**Figure 1. WT1 expression in human DC following  
adeno WT1 and Vaccinia WT1 infection**

**Control  
(uninfected  
human DC**



**Adeno WT1  
infected human  
DC**



**Vaccinia WT1  
infected human  
DC**

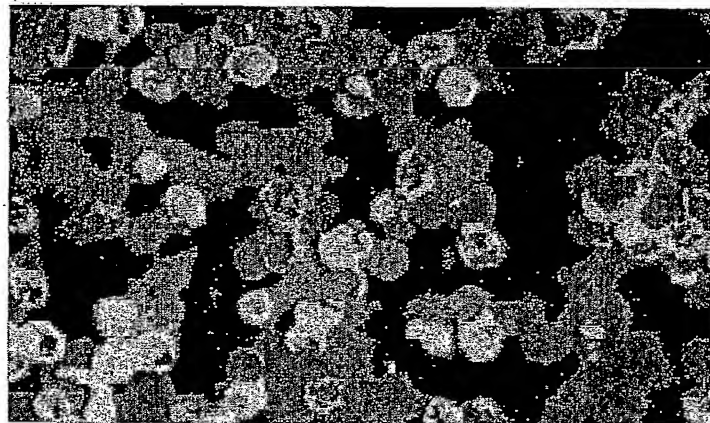
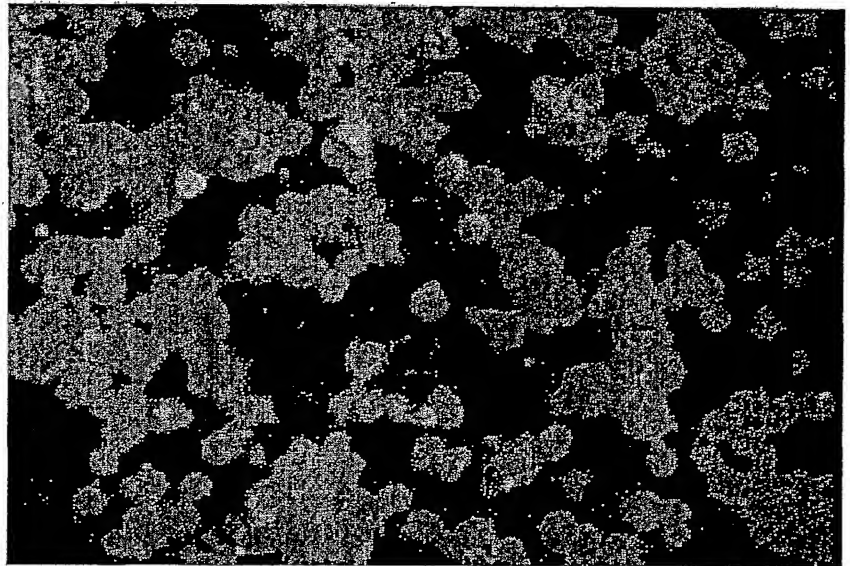


FIG. 22

**Figure 2. WT1 can be expressed reproducibly in human DC following adeno WT1 infection and is not induced by a control Adeno infection**

**Control  
(Adeno EGFP  
infected  
human DC)**



**Adeno WT1  
infected human  
DC**

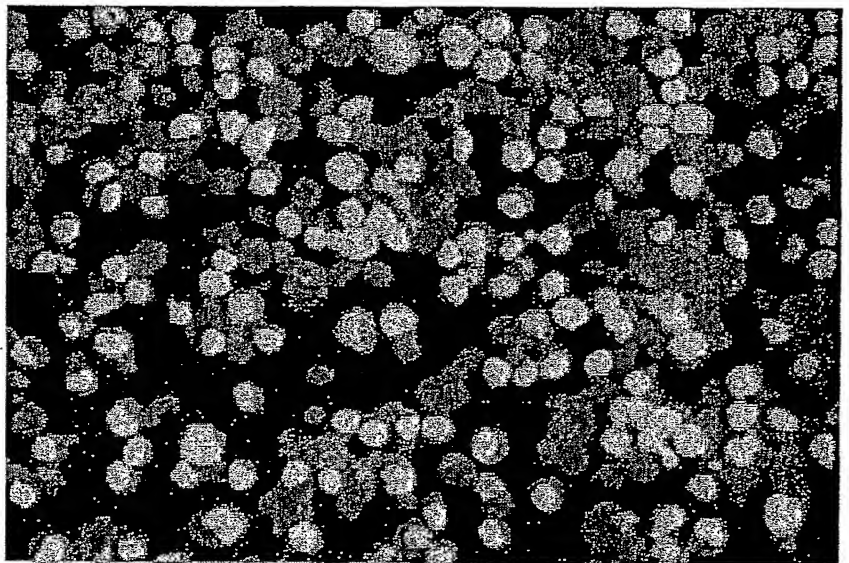
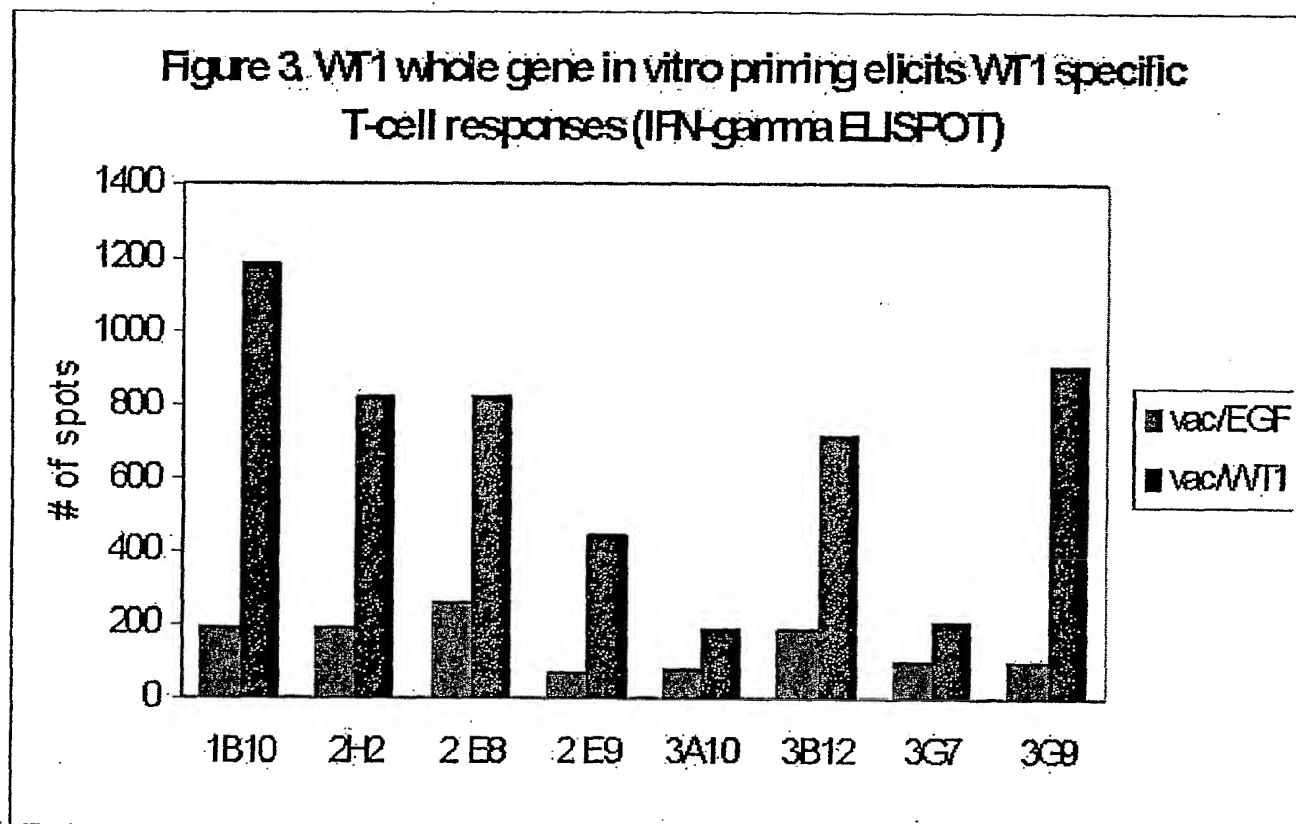


FIG. 23





## SEQUENCE LISTING

<110> Corixa Corporation  
Gaiger, Alexander  
McNeill, Patricia D.  
Smithgall, Molly  
Moulton, Gus  
Vedvick, Thomas S.  
Sleath, Paul R.  
Mossman, Sally  
Evans, Lawrence  
Spies, A. Gregory  
Boydston, Jeremy

<120> COMPOSITIONS AND METHODS FOR WT1  
SPECIFIC IMMUNOTHERAPY

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 <212> DNA  
 <213> Homo sapiens

<400> 328  
 atgcagcatc accaccatca ccacatgagc gataaaatta ttcacctgac tgacgacagt 60  
 tttgacacgg atgtactcaa agcggacggg gcgatcctcg tcgatttctg ggcagagtgg 120  
 tgcggtccgt gcaaaatgat cgccccgatt ctggatgaaa tcgctgacga atatcagggc 180  
 aaactgaccg ttgcaaaaact gaacatcgat caaaaccctg gcaactgcgc gaaatatggc 240  
 atccgtggta tcccgactct gctgctgttc aaaaacgggtg aagtggcggc aaccaaagtg 300  
 ggtgcactgt ctaaagggtca gttgaaagag ttctcgacg ctaacctggc cggttctgg 360  
 tctggccata tgcagcatca ccaccatcac cactgttcta tcgaaggctc tgctagctct 420  
 ggtggcagcg gtctggttcc gcgtggtagc tctggttcgg gggacgacga cgacaaatct 480  
 agtaggggct ccgacgttct tgacctgaac gcaactgtgc cggcagttcc gtccctgggt 540  
 ggtgggtggtg gttgcgcact gccggttagc ggtgcagcac agtgggctcc gggttctgg 600  
 ttgcacccgc cgggtgcatc cgcatacggg tccctgggtg gtccggcacc gccgcccggc 660  
 ccgcccgcgc cgcgcggcgc gccgcgcgac tccttcatca aacaggaacc gagctgggg 720  
 ggtgcagaac cgcacgaaga acagtgcctg agcgcattca ccgttcaact ctccggccag 780  
 ttcaactggc cagccggagc ctgtcgctac gggcccttct gtccctctcc gccagccag 840  
 gcgtcatccg gccaggccag gatgtttcct aacgcgccct acctgccag ctgcctcgag 900  
 agccagcccgc ctatttcgcaa tcagggttac agcaggtca ccttcgacgg gacgcccagc 960  
 tacggtcaca cgcctctgca ccatgcggcg cagttcccca accactcatt caagcatgag 1020  
 gatcccatgg gccagcaggg ctgcgtgggt gacgagcagt actcgggtgc gcccccgg 1080  
 tatggctgcc acacccccac cgacagctgc accggcagcc aggttttgc gctgaggacg 1140  
 ccctacagca gtgacaattt ataccaaagc acatcccagc ttgaatgcat gacctggaat 1200  
 cagatgaact taggagccac cttaaagggc tga 1233

<210> 329  
 <211> 1776  
 <212> DNA  
 <213> Homo sapiens

<400> 329  
 atgcagcatc accaccatca ccacatgagc gataaaatta ttcacctgac tgacgacagt 60  
 tttgacacgg atgtactcaa agcggacggg gcgatcctcg tcgatttctg ggcagagtgg 120  
 tgccgtccgt gcaaaatgat cgccccgatt ctggatgaaa tcgctgacga atatcagggc 180  
 aaactgaccg ttgcaaaact gaacatcgat caaaaccctg gcaactgcgc gaaatatggc 240  
 atccgtggta tcccgaactct gctgctgttc aaaaacgggtg aagtggcggc aaccaaagtg 300  
 ggtgcactgt ctaaagggtca gttgaaagag ttcctcgacg ctaacctggc cggttctggg 360  
 tctggccata tgcagcatca ccaccatcac cactgtgtct tgaagggtcg tgctagctct 420  
 ggtggcagcg gtctggttcc gctggttagc tctggttcgg gggacgacga cgacaaatct 480  
 agtaggatgg gctccgacgt tcgtgacctg aacgcactgc tgccggcagt tccgtccctg 540  
 ggtggtgggtg gtggttgccg actgccggtt agcggtgacg cacagtgggc tccggttctg 600  
 gaacttgcac cgccgggtgc atccgcatac ggttccctgg gtggtccggc accgccggc 660  
 gcaccgccgc cgccgccgcc gccgccgccg cactccttca tcaaacagga accgagctgg 720  
 ggtggtgcag aaccgcacga agaacagtgc ctgagcgcat tcaccgttca cttctccggc 780  
 cagttcactg gcacagccgg agcctgtcgc tacgggccct tcggtcctcc tccgcccagc 840  
 caggcgtcat ccggccaggc caggatgttt cctaaccgcg cctacctgcc cagctgcctc 900  
 gagagccagc ccgctattcg caatcagggt tacagcacgg tcaccttoga cgggacgccc 960  
 agctacggtc acacgccctc gcaccatgcg gcgcagttcc ccaaccactc attcaagcat 1020  
 gaggatccca tgggccagca gggctcgctg ggtgagcagc agtactcggg gccgcccccg 1080  
 gtctatggct gccacacccc caccgacagc tgcaccggca gccaggcttt gctgctgagg 1140  
 acgccctaca gcagtgacaa tttataccaa atgacatccc agcttgaatg catgacctgg 1200  
 aatcagatga acttaggagc caccttaaag ggccacagca cagggtacga gagcgataac 1260  
 cacacaacgc ccactcctct cgagcccaa tacagaatac acacgcacgg tgtcttcaga 1320  
 ggcattcagg atgtgcgacg tgtgcctgga gtagccccga ctcttgtagc gtcggcatct 1380  
 gagaccagtg agaaaacgcc cttcatgtgt gcttaccagc gctgcaataa gagatatttt 1440  
 aagctgtccc acttacagat gcacagcagg aagcacactg gtgagaaacc ataccagtgt 1500  
 gacttcaagg actgtgaacg aaggtttttt cgttcagacc agctcaaaag acaccaaagg 1560  
 agacatacag gtgtgaaacc attccagtgt aaaacttgtc agcgaaagtt ctcccgggtc 1620  
 gaccacctga agaccacac caggactcat acaggtgaaa agcccttcag ctgtcgggtg 1680  
 ccaagttgtc agaaaaagtt tgcccggtca gatgaattag tccgccatca caacatgcat 1740  
 cagagaaaca tgaccaaaact ccagctggcg ctttga 1776

<210> 330  
 <211> 771  
 <212> DNA  
 <213> Homo sapiens

<400> 330  
 atgcagcatc accaccatca ccacggctcc gacgttcgtg acctgaacgc actgctgccg 60  
 gcagttccgt ccctgggtgg tgggtggtgg tgcgcactgc cggtttagcg tgcagcacag 120  
 tgggtccgg ttctggactt cgcaccgccg ggtgcatccg catacggttc cctgggtggg 180  
 ccggcacccg cgccggcacc gccgccgccg ccgccgccgc cgccgcactc cttcatcaaa 240  
 caggaaccga gctgggggtg tgcagaaccg cacgaagaac agtgccctgag cgcattcacc 300  
 gttcacttct ccggccagtt cactggcaca gccggagcct gtcgctacgg gcccttcggg 360  
 cctcccgcgc ccagccaggc gtcattccggc caggccagga tgtttcctaa cgcgccctac 420  
 ctgcccagct gcctcgagag ccagcccgtc attcgcaatc agggttacag cacggtcacc 480  
 ttcgacggga cgcccagcta cggtcacacg ccctgcacc atgcggcgca gttccccaac 540  
 cactcattca agcatgagga tcccatgggc cagcagggtc cgctgggtga gcagcagtac 600  
 tcggtgcgcg ccccggtcta tgggtgccac acccccaccg acagctgcac cggcagccag 660  
 gctttgtctg tgaggacgcc ctacagcagt gacaatttat accaaatgac atcccagctt 720  
 gaatgcatga cctggaatca gatgaactta ggagccacct taaagggtcg a 771

<210> 331

```
<211> 567
<212> DNA
<213> Homo sapiens
```

<400> 331						
atgcagcatc	accaccatca	ccaccacagc	acaggggtacg	agagcgataa	ccacacaacg	60
cccatcctct	gcgagagcca	atacagaata	cacacgcacg	gtgtcttcag	aggcattcag	120
gatgtgcgac	gtgtgccttg	agtagccccg	actcttgtac	ggtcgggcac	tgagaccagt	180
gagaaacgcc	ccttcattgt	tgcttaccga	ggctgcaata	agagatatatt	taagctgtcc	240
cacttacaga	tgcacagcag	gaagcacact	ggtgagaaac	cataccagtg	tgacttcaag	300
gactgtgaac	gaagggtttt	tcgttcagac	cagctcaaaa	gacaccaaaag	gagacataca	360
ggtgtgaaac	cattccagtg	taaaacttgt	cagcgaaagt	tctcccggtc	cgaccacctg	420
aagaccacca	ccaggactca	tacaggtgaa	aagcccttca	gctgtcggtg	gccaaagtgt	480
cagaaaaagt	ttgcccggtc	agatgaatta	gtccgccatc	acaacatgca	tcagagaaac	540
atgaccaaac	tccagctggc	gctttga				567

```
<210> 332
<211> 342
<212> PRT
<213> Homo sapiens
```

<400>	332														
Met	Gln	His	His	His	His	His	His	Met	Ser	Asp	Lys	Ile	Ile	His	Leu
				5					10					15	
Thr	Asp	Asp	Ser	Phe	Asp	Thr	Asp	Val	Leu	Lys	Ala	Asp	Gly	Ala	Ile
			20					25					30		
Leu	Val	Asp	Phe	Trp	Ala	Glu	Trp	Cys	Gly	Pro	Cys	Lys	Met	Ile	Ala
		35					40					45			
Pro	Ile	Leu	Asp	Glu	Ile	Ala	Asp	Glu	Tyr	Gln	Gly	Lys	Leu	Thr	Val
	50					55					60				
Ala	Lys	Leu	Asn	Ile	Asp	Gln	Asn	Pro	Gly	Thr	Ala	Pro	Lys	Tyr	Gly
	65				70					75					80
Ile	Arg	Gly	Ile	Pro	Thr	Leu	Leu	Leu	Phe	Lys	Asn	Gly	Glu	Val	Ala
				85					90					95	
Ala	Thr	Lys	Val	Gly	Ala	Leu	Ser	Lys	Gly	Gln	Leu	Lys	Glu	Phe	Leu
			100					105					110		
Asp	Ala	Asn	Leu	Ala	Gly	Ser	Gly	Ser	Gly	His	Met	Gln	His	His	His
		115					120					125			
His	His	His	Val	Ser	Ile	Glu	Gly	Arg	Ala	Ser	Ser	Gly	Gly	Ser	Gly
	130					135					140				
Leu	Val	Pro	Arg	Gly	Ser	Ser	Gly	Ser	Gly	Asp	Asp	Asp	Asp	Lys	Ser
145					150					155					160
Ser	Arg	His	Ser	Thr	Gly	Tyr	Glu	Ser	Asp	Asn	His	Thr	Thr	Pro	Ile
				165					170					175	
Leu	Cys	Gly	Ala	Gln	Tyr	Arg	Ile	His	Thr	His	Gly	Val	Phe	Arg	Gly
			180					185					190		
Ile	Gln	Asp	Val	Arg	Arg	Val	Pro	Gly	Val	Ala	Pro	Thr	Leu	Val	Arg
		195					200					205			
Ser	Ala	Ser	Glu	Thr	Ser	Glu	Lys	Arg	Pro	Phe	Met	Cys	Ala	Tyr	Pro
	210					215					220				
Gly	Cys	Asn	Lys	Arg	Tyr	Phe	Lys	Leu	Ser	His	Leu	Gln	Met	His	Ser
225				230						235				240	
Arg	Lys	His	Thr	Gly	Glu	Lys	Pro	Tyr	Gln	Cys	Asp	Phe	Lys	Asp	Cys
				245					250					255	
Glu	Arg	Arg	Phe	Phe	Arg	Ser	Asp	Gln	Leu	Lys	Arg	His	Gln	Arg	Arg





```

Ile Arg Asn Gln Gly Tyr Ser Thr Val Thr Phe Asp Gly Thr Pro Ser
305                      310                      315                      320
Tyr Gly His Thr Pro Ser His His Ala Ala Gln Phe Pro Asn His Ser
                      325                      330                      335
Phe Lys His Glu Asp Pro Met Gly Gln Gly Ser Leu Gly Glu Gln
                      340                      345                      350
Gln Tyr Ser Val Pro Pro Pro Val Tyr Gly Cys His Thr Pro Thr Asp
                      355                      360                      365
Ser Cys Thr Gly Ser Gln Ala Leu Leu Leu Arg Thr Pro Tyr Ser Ser
                      370                      375                      380
Asp Asn Leu Tyr Gln Met Thr Ser Gln Leu Glu Cys Met Thr Trp Asn
385                      390                      395                      400
Gln Met Asn Leu Gly Ala Thr Leu Lys Gly
                      405                      410

```

&lt;210&gt; 334

&lt;211&gt; 591

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 334

```

Met Gln His His His His His His Met Ser Asp Lys Ile Ile His Leu
                      5                      10                      15
Thr Asp Asp Ser Phe Asp Thr Asp Val Leu Lys Ala Asp Gly Ala Ile
                      20                      25                      30
Leu Val Asp Phe Trp Ala Glu Trp Cys Gly Pro Cys Lys Met Ile Ala
                      35                      40                      45
Pro Ile Leu Asp Glu Ile Ala Asp Glu Tyr Gln Gly Lys Leu Thr Val
                      50                      55                      60
Ala Lys Leu Asn Ile Asp Gln Asn Pro Gly Thr Ala Pro Lys Tyr Gly
65                      70                      75                      80
Ile Arg Gly Ile Pro Thr Leu Leu Leu Phe Lys Asn Gly Glu Val Ala
                      85                      90                      95
Ala Thr Lys Val Gly Ala Leu Ser Lys Gly Gln Leu Lys Glu Phe Leu
                      100                     105                     110
Asp Ala Asn Leu Ala Gly Ser Gly Ser Gly His Met Gln His His His
                      115                     120                     125
His His His Val Ser Ile Glu Gly Arg Ala Ser Ser Gly Gly Ser Gly
130                     135                     140
Leu Val Pro Arg Gly Ser Ser Gly Ser Gly Asp Asp Asp Lys Ser
145                     150                     155                     160
Ser Arg Met Gly Ser Asp Val Arg Asp Leu Asn Ala Leu Leu Pro Ala
                      165                     170                     175
Val Pro Ser Leu Gly Gly Gly Gly Gly Cys Ala Leu Pro Val Ser Gly
                      180                     185                     190
Ala Ala Gln Trp Ala Pro Val Leu Asp Phe Ala Pro Pro Gly Ala Ser
195                     200                     205
Ala Tyr Gly Ser Leu Gly Gly Pro Ala Pro Pro Pro Ala Pro Pro Pro
210                     215                     220
Pro Pro Pro Pro Pro His Ser Phe Ile Lys Gln Glu Pro Ser Trp
225                     230                     235                     240
Gly Gly Ala Glu Pro His Glu Glu Gln Cys Leu Ser Ala Phe Thr Val
                      245                     250                     255
His Phe Ser Gly Gln Phe Thr Gly Thr Ala Gly Ala Cys Arg Tyr Gly
260                     265                     270
Pro Phe Gly Pro Pro Pro Pro Ser Gln Ala Ser Ser Gly Gln Ala Arg
275                     280                     285

```

```

Met Phe Pro Asn Ala Pro Tyr Leu Pro Ser Cys Leu Glu Ser Gln Pro
 290                295                300
Ala Ile Arg Asn Gln Gly Tyr Ser Thr Val Thr Phe Asp Gly Thr Pro
305                310                315
Ser Tyr Gly His Thr Pro Ser His His Ala Ala Gln Phe Pro Asn His
                325                330                335
Ser Phe Lys His Glu Asp Pro Met Gly Gln Gln Gly Ser Leu Gly Glu
                340                345                350
Gln Gln Tyr Ser Val Pro Pro Pro Val Tyr Gly Cys His Thr Pro Thr
                355                360                365
Asp Ser Cys Thr Gly Ser Gln Ala Leu Leu Leu Arg Thr Pro Tyr Ser
                370                375                380
Ser Asp Asn Leu Tyr Gln Met Thr Ser Gln Leu Glu Cys Met Thr Trp
385                390                395
Asn Gln Met Asn Leu Gly Ala Thr Leu Lys Gly His Ser Thr Gly Tyr
                405                410                415
Glu Ser Asp Asn His Thr Thr Pro Ile Leu Cys Gly Ala Gln Tyr Arg
                420                425                430
Ile His Thr His Gly Val Phe Arg Gly Ile Gln Asp Val Arg Arg Val
                435                440                445
Pro Gly Val Ala Pro Thr Leu Val Arg Ser Ala Ser Glu Thr Ser Glu
                450                455                460
Lys Arg Pro Phe Met Cys Ala Tyr Pro Gly Cys Asn Lys Arg Tyr Phe
465                470                475
Lys Leu Ser His Leu Gln Met His Ser Arg Lys His Thr Gly Glu Lys
                485                490                495
Pro Tyr Gln Cys Asp Phe Lys Asp Cys Glu Arg Arg Phe Phe Arg Ser
                500                505                510
Asp Gln Leu Lys Arg His Gln Arg Arg His Thr Gly Val Lys Pro Phe
                515                520                525
Gln Cys Lys Thr Cys Gln Arg Lys Phe Ser Arg Ser Asp His Leu Lys
                530                535                540
Thr His Thr Arg Thr His Thr Gly Glu Lys Pro Phe Ser Cys Arg Trp
545                550                555
Pro Ser Cys Gln Lys Lys Phe Ala Arg Ser Asp Glu Leu Val Arg His
                565                570                575
His Asn Met His Gln Arg Asn Met Thr Lys Leu Gln Leu Ala Leu
                580                585                590

```

&lt;210&gt; 335

&lt;211&gt; 256

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 335

```

Met Gln His His His His His His Gly Ser Asp Val Arg Asp Leu Asn
                    5                    10                    15
Ala Leu Leu Pro Ala Val Pro Ser Leu Gly Gly Gly Gly Gly Cys Ala
                20                25                30
Leu Pro Val Ser Gly Ala Ala Gln Trp Ala Pro Val Leu Asp Phe Ala
                35                40                45
Pro Pro Gly Ala Ser Ala Tyr Gly Ser Leu Gly Gly Pro Ala Pro Pro
                50                55                60
Pro Ala Pro Pro Pro Pro Pro Pro Pro Pro Pro His Ser Phe Ile Lys
                65                70                75                80
Gln Glu Pro Ser Trp Gly Gly Ala Glu Pro His Glu Glu Gln Cys Leu

```

```
<210> 336
<211> 188
<212> PRT
<213> Homo sapiens
```

Met	Gln	His	His	His	His	His	His	His	Ser	Thr	Gly	Tyr	Glu	Ser	Asp
				5					10					15	
Asn	His	Thr	Thr	Pro	Ile	Leu	Cys	Gly	Ala	Gln	Tyr	Arg	Ile	His	Thr
			20					25					30		
His	Gly	Val	Phe	Arg	Gly	Ile	Gln	Asp	Val	Arg	Arg	Val	Pro	Gly	Val
		35					40					45			
Ala	Pro	Thr	Leu	Val	Arg	Ser	Ala	Ser	Glu	Thr	Ser	Glu	Lys	Arg	Pro
	50					55					60				
Phe	Met	Cys	Ala	Tyr	Pro	Gly	Cys	Asn	Lys	Arg	Tyr	Phe	Lys	Leu	Ser
65				70					75					80	
His	Leu	Gln	Met	His	Ser	Arg	Lys	His	Thr	Gly	Glu	Lys	Pro	Tyr	Gln
				85				90					95		
Cys	Asp	Phe	Lys	Asp	Cys	Glu	Arg	Arg	Phe	Phe	Arg	Ser	Asp	Gln	Leu
			100					105				110			
Lys	Arg	His	Gln	Arg	Arg	His	Thr	Gly	Val	Lys	Pro	Phe	Gln	Cys	Lys
		115					120				125				
Thr	Cys	Gln	Arg	Lys	Phe	Ser	Arg	Ser	Asp	His	Leu	Lys	Thr	His	Thr
	130					135					140				
Arg	Thr	His	Thr	Gly	Glu	Lys	Pro	Phe	Ser	Cys	Arg	Trp	Pro	Ser	Cys
145				150						155				160	
Gln	Lys	Lys	Phe	Ala	Arg	Ser	Asp	Glu	Leu	Val	Arg	His	His	Asn	Met
				165					170					175	
His	Gln	Arg	Asn	Met	Thr	Lys	Leu	Gln	Leu	Ala	Leu				
			180					185							

```
<210> 337
<211> 324
<212> DNA
<213> Homo sapiens
```

<400> 337  
 atgcagcatc accaccatca ccacgggttc gacgtgcggg acctgaacgc actgctgccg 60  
 gcagttccat ccctgggtgg cgggtggaggc tgcgcactgc cggttagcgg tgcagcacag 120  
 tgggctccag ttctggactt cgcaccgctt ggtgcatccg catacggttc cctgggtggg 180  
 ccagcacctc cgcccgaac gccccaccc cctccaccgc ccccgcactc cttcatcaaa 240  
 caggaacctc gctgggtgg tgcagaaccg cacgaagaac agtgcctgag cgcattctga 300  
 gaattctgca gatatccatc acac 324

<210> 338  
 <211> 462  
 <212> DNA  
 <213> Homo sapiens

<400> 338  
 atgcagcatc accaccatca ccaccacgaa gaacagtgcc tgagcgcatt caccgttcac 60  
 ttctccggcc agttcactgg cacagccgga gcctgtcgtt acggggccctt cggtcctcct 120  
 ccgcccagcc aggcgtcatc cggccaggcc aggatgtttc ctaacgcgcc ctacctgcc 180  
 agctgcctcg agagccagcc cgctattcgc aatcaggggtt acagcacggg caccttcgac 240  
 gggacgcccc gctacggtca cagccctcgc caccatgcgg cgcagttccc caaccactca 300  
 ttcaagcatg aggatcccat gggccagcag ggctcgtctg gtgagcagca gtactcgggtg 360  
 ccgcccccg tctatggctg ccacaccccc accgacagct gcaccggcag ccaggctttg 420  
 ctgctgagga cgccctacag cagtgacaat ttatactgat ga 462

<210> 339  
 <211> 405  
 <212> DNA  
 <213> Homo sapiens

<400> 339  
 atgcagcatc accaccatca ccaccaggct ttgctgctga ggacgcccta cagcagtgc 60  
 aatttatacc aaatgacatc ccagcttgaa tgcattgacct ggaatcagat gaacttagga 120  
 gccaccttaa agggccacag cacagggtac gagagcgata accacacaac gccatcctc 180  
 tgcggagccc aatacagaat acacacgcac ggtgtcttca gaggcattca ggatgtgcga 240  
 cgtgtgcctg gagtagcccc gactcttgta cggtcggcat ctgagaccag tgagaaacgc 300  
 cccttcatgt gtgcttacc caggctgcaat aagagatatt ttaagctgtc ccacttacag 360  
 atgcacagca ggaagcacac tgggtgagaaa ccataccagt gatga 405

<210> 340  
 <211> 339  
 <212> DNA  
 <213> Homo sapiens

<400> 340  
 atgcagcatc accaccatca ccaccacagc aggaagcaca ctggtgagaa accataccag 60  
 tgtgacttca aggactgtga acgaaggttt tttcgttcag accagctcaa aagacaccaa 120  
 aggagacata caggtgtgaa accattccag tgtaaaactt gtcagcgaaa gttctcccg 180  
 tccgaccacc tgaagaccca caccaggact catacagggtg aaaagccctt cagctgtcgg 240  
 tggccaagtt gtcagaaaaa gtttgcccgg tcagatgaat tagtccgcca tcacaacatg 300  
 catcagagaa acatgaccaa actccagctg gcgctttga 339

<210> 341  
 <211> 1110  
 <212> DNA  
 <213> Homo sapiens

&lt;400&gt; 341

```

atgcagcatc accaccatca ccaccactcc ttcatcaaac aggaaccgag ctgggggtggt 60
gcagaaccgc acgaagaaca gtgcctgagc gcattcacccg ttcaacttctc cggccaggttc 120
actggcacag ccggagcctg tcgctacggg cccttcgggtc ctcctccgcc cagccaggcg 180
tcatccggcc aggcaggat gtttcctaac gcgccctacc tgcccagctg cctcgagagc 240
cagcccgccta ttcgcaatca ggggttacgc acgggtcacct tcgacgggac gccagctac 300
ggtcacacgc cctcgcacca tgcggcgcag ttccccaacc actcattcaa gcatgaggat 360
cccatgggcc agcagggctc gctgggtgag cagcagtact cggtgccgcc cccggtctat 420
ggctgccaca cccccaccga cagctgcacc ggcagccagg ctttgctgct gaggacgcc 480
tacagcagtg acaatttata ccaaatagaca tcccagcttg aatgcatgac ctggaatcag 540
atgaacttag gagccacctt aaagggccac agcacagggt acgagagcga taaccacaca 600
acgcccattcc tctgcggagc ccaatacaga atacacacgc acggtgtctt cagaggcatt 660
caggatgtgc gacgtgtgcc tggagtagcc ccgactcttg tacggtcggc atctgagacc 720
agtgagaaac gcccttcat gtgtgcttac ccaggctgca ataagagata ttttaagctg 780
tcccacttac agatgcacag caggaagcac actggtgaga aaccatacca gtgtgacttc 840
aaggactgtg aacgaagggt ttttcgttca gaccagctca aaagacacca aaggagacat 900
acaggtgtga aaccattcca gtgtaaaact tgtcagcgaa agttctcccg gtccgaccac 960
ctgaagaccc acaccaggac tcatacaggt gaaaagccct tcagctgtcg gtggccaagt 1020
tgtcagaaaa agtttgcccg gtcagatgaa ttagtccgcc atcacaacat gcatcagaga 1080
aacatgacca aactccagct ggcgcttga

```

&lt;210&gt; 342

&lt;211&gt; 99

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 342

```

Met Gln His His His His His Gly Ser Asp Val Arg Asp Leu Asn
                    5              10              15
Ala Leu Leu Pro Ala Val Pro Ser Leu Gly Gly Gly Gly Gly Cys Ala
                20              25              30
Leu Pro Val Ser Gly Ala Ala Gln Trp Ala Pro Val Leu Asp Phe Ala
                35              40              45
Pro Pro Gly Ala Ser Ala Tyr Gly Ser Leu Gly Gly Pro Ala Pro Pro
                50              55              60
Pro Ala Pro Pro Pro Pro Pro Pro Pro Pro Pro His Ser Phe Ile Lys
                65              70              75              80
Gln Glu Pro Ser Trp Gly Gly Ala Glu Pro His Glu Glu Gln Cys Leu
                85              90              95
Ser Ala Phe

```

&lt;210&gt; 343

&lt;211&gt; 152

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 343

```

Met Gln His His His His His His Glu Glu Gln Cys Leu Ser Ala
                    5              10              15
Phe Thr Val His Phe Ser Gly Gln Phe Thr Gly Thr Ala Gly Ala Cys
                20              25              30
Arg Tyr Gly Pro Phe Gly Pro Pro Pro Pro Ser Gln Ala Ser Ser Gly
                35              40              45
Gln Ala Arg Met Phe Pro Asn Ala Pro Tyr Leu Pro Ser Cys Leu Glu
                50              55              60
Ser Gln Pro Ala Ile Arg Asn Gln Gly Tyr Ser Thr Val Thr Phe Asp

```

```

      65              70              75              80
Gly Thr Pro Ser Tyr Gly His Thr Pro Ser His His Ala Ala Gln Phe
      85              90
Pro Asn His Ser Phe Lys His Glu Asp Pro Met Gly Gln Gln Gly Ser
      100            105            110
Leu Gly Glu Gln Gln Tyr Ser Val Pro Pro Pro Val Tyr Gly Cys His
      115            120            125
Thr Pro Thr Asp Ser Cys Thr Gly Ser Gln Ala Leu Leu Leu Arg Thr
      130            135            140
Pro Tyr Ser Ser Asp Asn Leu Tyr
145              150

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<210> 344  
 <211> 133  
 <212> PRT  
 <213> Homo sapiens

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<400> 344
Met Gln His His His His His His Gln Ala Leu Leu Leu Arg Thr Pro
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Tyr Ser Ser Asp Asn Leu Tyr Gln Met Thr Ser Gln Leu Glu Cys Met
      20            25            30
Thr Trp Asn Gln Met Asn Leu Gly Ala Thr Leu Lys Gly His Ser Thr
      35            40            45
Gly Tyr Glu Ser Asp Asn His Thr Thr Pro Ile Leu Cys Gly Ala Gln
      50            55            60
Tyr Arg Ile His Thr His Gly Val Phe Arg Gly Ile Gln Asp Val Arg
      65            70            75            80
Arg Val Pro Gly Val Ala Pro Thr Leu Val Arg Ser Ala Ser Glu Thr
      85            90            95
Ser Glu Lys Arg Pro Phe Met Cys Ala Tyr Pro Gly Cys Asn Lys Arg
      100           105           110
Tyr Phe Lys Leu Ser His Leu Gln Met His Ser Arg Lys His Thr Gly
      115           120           125
Glu Lys Pro Tyr Gln
130

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<210> 345  
 <211> 112  
 <212> PRT  
 <213> Homo sapiens

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<400> 345
Met Gln His His His His His His His Ser Arg Lys His Thr Gly Glu
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Lys Pro Tyr Gln Cys Asp Phe Lys Asp Cys Glu Arg Arg Phe Phe Arg
      20            25            30
Ser Asp Gln Leu Lys Arg His Gln Arg Arg His Thr Gly Val Lys Pro
      35            40            45
Phe Gln Cys Lys Thr Cys Gln Arg Lys Phe Ser Arg Ser Asp His Leu
      50            55            60
Lys Thr His Thr Arg Thr His Thr Gly Glu Lys Pro Phe Ser Cys Arg
      65            70            75            80
Trp Pro Ser Cys Gln Lys Lys Phe Ala Arg Ser Asp Glu Leu Val Arg
      85            90            95
His His Asn Met His Gln Arg Asn Met Thr Lys Leu Gln Leu Ala Leu

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100					105					110					
<210> 346															
<211> 369															
<212> PRT															
<213> Homo sapiens															
<400> 346															
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			20					25					30		
Thr	Val	His	Phe	Ser	Gly	Gln	Phe	Thr	Gly	Thr	Ala	Gly	Ala	Cys	Arg
		35					40					45			
Tyr	Gly	Pro	Phe	Gly	Pro	Pro	Pro	Pro	Ser	Gln	Ala	Ser	Ser	Gly	Gln
	50					55					60				
Ala	Arg	Met	Phe	Pro	Asn	Ala	Pro	Tyr	Leu	Pro	Ser	Cys	Leu	Glu	Ser
	65				70					75					80
Gln	Pro	Ala	Ile	Arg	Asn	Gln	Gly	Tyr	Ser	Thr	Val	Thr	Phe	Asp	Gly
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			100					105					110		
Asn	His	Ser	Phe	Lys	His	Glu	Asp	Pro	Met	Gly	Gln	Gln	Gly	Ser	Leu
		115					120					125			
Gly	Glu	Gln	Gln	Tyr	Ser	Val	Pro	Pro	Pro	Val	Tyr	Gly	Cys	His	Thr
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	145				150					155					160
Tyr	Ser	Ser	Asp	Asn	Leu	Tyr	Gln	Met	Thr	Ser	Gln	Leu	Glu	Cys	Met
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Thr	Trp	Asn	Gln	Met	Asn	Leu	Gly	Ala	Thr	Leu	Lys	Gly	His	Ser	Thr
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		195					200					205			
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	225				230					235					240
Ser	Glu	Lys	Arg	Pro	Phe	Met	Cys	Ala	Tyr	Pro	Gly	Cys	Asn	Lys	Arg
				245					250					255	
Tyr	Phe	Lys	Leu	Ser	His	Leu	Gln	Met	His	Ser	Arg	Lys	His	Thr	Gly
			260					265					270		
Glu	Lys	Pro	Tyr	Gln	Cys	Asp	Phe	Lys	Asp	Cys	Glu	Arg	Arg	Phe	Phe
		275					280					285			
Arg	Ser	Asp	Gln	Leu	Lys	Arg	His	Gln	Arg	Arg	His	Thr	Gly	Val	Lys
	290					295					300				
Pro	Phe	Gln	Cys	Lys	Thr	Cys	Gln	Arg	Lys	Phe	Ser	Arg	Ser	Asp	His
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<211> 29

<212> DNA

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<223> Primer

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29

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<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 354

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32

<210> 355

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 355

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34

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<211> 28

<212> DNA

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<223> Primer

<400> 357

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28

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<223> Primer

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<223> Primer

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<223> Primer

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33

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 <400> 376  
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 <210> 377  
 <211> 1292  
 <212> DNA  
 <213> Homo sapiens  
  
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 758  
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 gcaccgccgg gtgcatccgc acacggtccc ctgggtgggt cggcgccgcc gtcggcaccg 180  
 ccgccgccgc cgccgccgcc gccgcactcc ttcattcaaac agggaccgag ctgggggtggc 240  
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&lt;210&gt; 378

&lt;211&gt; 1291

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 378

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&lt;210&gt; 379

&lt;211&gt; 1281

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 379

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gcaccgccgg gcgcatccgc atacgattcc ctgggtggcc cggcaccgcc gccggcgccg 180

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&lt;210&gt; 380

&lt;211&gt; 3020

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 380

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&lt;210&gt; 381

&lt;211&gt; 1291

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 381

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&lt;210&gt; 382

&lt;211&gt; 1491

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 382

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&lt;210&gt; 383

&lt;211&gt; 1251

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 383

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ataatggcca acttctctgc tgctttctca gtgaactacg acaccaagag tggccccaag 180
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&lt;210&gt; 384

&lt;211&gt; 228

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 384

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cagaggttga	tctttgccgg	aaaacagctg	gaagatggtc	gtaccctgtc	tgactacaac	180
atccagaaag	agtcacacct	gcacctggta	ctccgtctca	gaggtggg		228

&lt;210&gt; 385

&lt;211&gt; 1515

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 385

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&lt;210&gt; 386

&lt;211&gt; 648

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 386

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&lt;210&gt; 387

&lt;211&gt; 1089

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 387

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&lt;210&gt; 388

&lt;211&gt; 1035

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 388

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&lt;210&gt; 389

&lt;211&gt; 1263

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 389

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&lt;210&gt; 390

&lt;211&gt; 1707

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 390

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atcgggcagg cgatggcgat cgcggggccag atcaagcttc ccaccgttca tatcgggcct 120
accgccttcc tcggcttggg tgttgctgac aacaacggca acggcgacag agtccaacgc 180
gtggtcggga gcgctccggc ggcaagtctc ggcatctcca ccggcgacgt gatcaccgcg 240
gtcgacggcg ctccgatcaa ctcgccacc gcgatggcgg acgcgttaa cgggcatcat 300
cccgtgacg tcatctcggg gacctggcaa accaagtccg gcggcacgcg tacagggaac 360
gtgacattgg ccgagggacc ccgggccgaa ttcccgtgg tgccgcgcgg cagcccgatg 420
ggctccgacg ttcgggacct gaacgcactg ctgccggcag ttccgtccct gggtggtggg 480
ggtggttgcg cactgccggg tagcggtgca gcacagtggg ctccggttct ggacttcgca 540
ccgccgggtg catccgcata cggttccctg ggtgggtccgg caccgcgcgc ggcaccgcgc 600
ccgccgcgc cgccgcgcgc gcaactcttc atcaaacagg aaccgagctg ggtggtgca 660
gaaccgcacg aagaacagtg cctgagcgca ttcacgttc acttctccgg ccagttcact 720
ggcacagccg gaggctgtcg ctacgggcc ttcggctctc ctccgcccag ccaggcgtca 780
tcgggccagg ccaggatggt tcctaacgcg ccctacctgc ccagctgcct cgagagccag 840
ccgctatttc gcaatcaggg ttacagcag gtcaccttcg acgggacgcc cagctacggg 900
cacacgccct cgcaccatgc ggcgcagttc cccaaccact cattcaagca tgaggatccc 960
atgggccagc agggctcgct gggtgagcag cagtactcgg tgccgcccc ggtctatggc 1020
tgccacaccc ccaccgacag ctgcaccggc agccaggctt tgctgctgag gacgccctac 1080
agcagtgaca atttatacca aatgacatcc cagcttgaat gcatgacctg gaatcagatg 1140
aacttaggag ccaccttaaa gggccacagc acagggtaac agagcgataa ccacacaacg 1200
ccatcctct gcggagccca atacagaata cacacgcacg gtgtcttcag aggcattcag 1260
gatgtgcgac gtgtgcctgg agtagcccc actcttgtac ggtcggcatc tgagaccagt 1320
gagaaacgcc ccttcatgtg tgcttaccga ggctgcaata agagatattt taagctgtcc 1380
cacttacaga tgcacagcag gaagcacact ggtgagaaac cataccagtg tgacttcaag 1440
gactgtgaac gaagggtttt tcgttcagac cagctcaaaa gacaccaaag gagacataca 1500
ggtgtgaaac cattccagtg taaaacttgt cagcgaaagt tctcccgtc cgaccacctg 1560

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Ser	Val	Pro	Pro 260	Pro	Val	Tyr	Gly	Cys 265	His	Thr	Pro	Thr	Asp 270	Ser	Cys
Thr	Gly	Ser 275	Gln	Ala	Leu	Leu	Leu 280	Arg	Thr	Pro	Tyr	Ser 285	Ser	Asp	Asn
Leu	Tyr 290	Gln	Met	Thr	Ser	Gln 295	Leu	Glu	Cys	Met	Thr 300	Trp	Asn	Gln	Met
Asn 305	Leu	Gly	Ala	Thr	Leu 310	Lys	Gly	His	Ser	Thr 315	Gly	Tyr	Glu	Ser	Asp 320
Asn	His	Thr	Thr	Pro 325	Ile	Leu	Cys	Gly	Ala 330	Gln	Tyr	Arg	Ile	His 335	Thr
His	Gly	Val	Phe 340	Arg	Gly	Ile	Gln								

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<210> 392
<211> 568
<212> PRT
<213> Homo sapiens
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<400> 392																	
Met	Thr	Ala	Ala	Ser	Asp	Asn	Phe	Gln	Leu	Ser	Gln	Gly	Gly	Gln	Gly		
				5					10					15			
Phe	Ala	Ile	Pro	Ile	Gly	Gln	Ala	Met	Ala	Ile	Ala	Gly	Gln	Ile	Lys		
				20					25					30			
Leu	Pro	Thr	Val	His	Ile	Gly	Pro	Thr	Ala	Phe	Leu	Gly	Leu	Gly	Val		
				35					40					45			
Val	Asp	Asn	Asn	Gly	Asn	Gly	Ala	Arg	Val	Gln	Arg	Val	Val	Gly	Ser		
				50					55					60			
Ala	Pro	Ala	Ala	Ser	Leu	Gly	Ile	Ser	Thr	Gly	Asp	Val	Ile	Thr	Ala		
65					70					75					80		
Val	Asp	Gly	Ala	Pro	Ile	Asn	Ser	Ala	Thr	Ala	Met	Ala	Asp	Ala	Leu		
				85					90					95			
Asn	Gly	His	His	Pro	Gly	Asp	Val	Ile	Ser	Val	Thr	Trp	Gln	Thr	Lys		
				100					105					110			
Ser	Gly	Gly	Thr	Arg	Thr	Gly	Asn	Val	Thr	Leu	Ala	Glu	Gly	Pro	Pro		
				115					120					125			
Ala	Glu	Phe	Pro	Leu	Val	Pro	Arg	Gly	Ser	Pro	Met	Gly	Ser	Asp	Val		
				130					135					140			
Arg	Asp	Leu	Asn	Ala	Leu	Leu	Pro	Ala	Val	Pro	Ser	Leu	Gly	Gly	Gly		
145					150					155					160		
Gly	Gly	Cys	Ala	Leu	Pro	Val	Ser	Gly	Ala	Ala	Gln	Trp	Ala	Pro	Val		
				165					170					175			

Leu Asp Phe Ala Pro Pro Gly Ala Ser Ala Tyr Gly Ser Leu Gly Gly  
 180 185 190  
 Pro Ala Pro Pro Pro Ala Pro Pro Pro Pro Pro Pro Pro Pro His  
 195 200 205  
 Ser Phe Ile Lys Gln Glu Pro Ser Trp Gly Gly Ala Glu Pro His Glu  
 210 215 220  
 Glu Gln Cys Leu Ser Ala Phe Thr Val His Phe Ser Gly Gln Phe Thr  
 225 230 235 240  
 Gly Thr Ala Gly Ala Cys Arg Tyr Gly Pro Phe Gly Pro Pro Pro Pro  
 245 250 255  
 Ser Gln Ala Ser Ser Gly Gln Ala Arg Met Phe Pro Asn Ala Pro Tyr  
 260 265 270  
 Leu Pro Ser Cys Leu Glu Ser Gln Pro Ala Ile Arg Asn Gln Gly Tyr  
 275 280 285  
 Ser Thr Val Thr Phe Asp Gly Thr Pro Ser Tyr Gly His Thr Pro Ser  
 290 295 300  
 His His Ala Ala Gln Phe Pro Asn His Ser Phe Lys His Glu Asp Pro  
 305 310 315 320  
 Met Gly Gln Gln Gly Ser Leu Gly Glu Gln Gln Tyr Ser Val Pro Pro  
 325 330 335  
 Pro Val Tyr Gly Cys His Thr Pro Thr Asp Ser Cys Thr Gly Ser Gln  
 340 345 350  
 Ala Leu Leu Leu Arg Thr Pro Tyr Ser Ser Asp Asn Leu Tyr Gln Met  
 355 360 365  
 Thr Ser Gln Leu Glu Cys Met Thr Trp Asn Gln Met Asn Leu Gly Ala  
 370 375 380  
 Thr Leu Lys Gly His Ser Thr Gly Tyr Glu Ser Asp Asn His Thr Thr  
 385 390 395 400  
 Pro Ile Leu Cys Gly Ala Gln Tyr Arg Ile His Thr His Gly Val Phe  
 405 410 415  
 Arg Gly Ile Gln Asp Val Arg Arg Val Pro Gly Val Ala Pro Thr Leu  
 420 425 430  
 Val Arg Ser Ala Ser Glu Thr Ser Glu Lys Arg Pro Phe Met Cys Ala  
 435 440 445  
 Tyr Pro Gly Cys Asn Lys Arg Tyr Phe Lys Leu Ser His Leu Gln Met  
 450 455 460  
 His Ser Arg Lys His Thr Gly Glu Lys Pro Tyr Gln Cys Asp Phe Lys  
 465 470 475 480  
 Asp Cys Glu Arg Arg Phe Phe Arg Ser Asp Gln Leu Lys Arg His Gln



485                      490                      495  
 Arg Arg His Thr Gly Val Lys Pro Phe Gln Cys Lys Thr Cys Gln Arg  
                     500                      505                      510  
 Lys Phe Ser Arg Ser Asp His Leu Lys Thr His Thr Arg Thr His Thr  
                     515                      520                      525  
 Gly Glu Lys Pro Phe Ser Cys Arg Trp Pro Ser Cys Gln Lys Lys Phe  
                     530                      535                      540  
 Ala Arg Ser Asp Glu Leu Val Arg His His Asn Met His Gln Arg Asn  
 545                      550                      555                      560  
 Met Thr Lys Leu Gln Leu Ala Leu  
                     565

&lt;210&gt; 393

&lt;211&gt; 420

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 393

Met Thr Ala Ala Ser Asp Asn Phe Gln Leu Ser Gln Gly Gly Gln Gly  
                     5                      10                      15  
 Phe Ala Ile Pro Ile Gly Gln Ala Met Ala Ile Ala Gly Gln Ile Lys  
                     20                      25                      30  
 Leu Pro Thr Val His Ile Gly Pro Thr Ala Phe Leu Gly Leu Gly Val  
                     35                      40                      45  
 Val Asp Asn Asn Gly Asn Gly Ala Arg Val Gln Arg Val Val Gly Ser  
                     50                      55                      60  
 Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr Gly Asp Val Ile Thr Ala  
                     65                      70                      75                      80  
 Val Asp Gly Ala Pro Ile Asn Ser Ala Thr Ala Met Ala Asp Ala Leu  
                     85                      90                      95  
 Asn Gly His His Pro Gly Asp Val Ile Ser Val Thr Trp Gln Thr Lys  
                     100                      105                      110  
 Ser Gly Gly Thr Arg Thr Gly Asn Val Thr Leu Ala Glu Gly Pro Pro  
                     115                      120                      125  
 Ala Glu Phe Pro Leu Val Pro Arg Gly Ser Pro Met Gly Ser Asp Val  
                     130                      135                      140  
 Arg Asp Leu Asn Ala Leu Leu Pro Ala Val Pro Ser Leu Gly Gly Gly  
 145                      150                      155                      160  
 Gly Gly Cys Ala Leu Pro Val Ser Gly Ala Ala Gln Trp Ala Pro Val  
                     165                      170                      175  
 Leu Asp Phe Ala Pro Pro Gly Ala Ser Ala Tyr Gly Ser Leu Gly Gly

180					185					190				
Pro	Ala	Pro	Pro	Pro	Ala	Pro	Pro	Pro	Pro	Pro	Pro	Pro	Pro	His
	195					200					205			
Ser	Phe	Ile	Lys	Gln	Glu	Pro	Ser	Trp	Gly	Gly	Ala	Glu	Pro	His
	210					215					220			
Glu	Gln	Cys	Leu	Ser	Ala	Phe	Thr	Val	His	Phe	Ser	Gly	Gln	Phe
	225					230					235			240
Gly	Thr	Ala	Gly	Ala	Cys	Arg	Tyr	Gly	Pro	Phe	Gly	Pro	Pro	Pro
				245					250					255
Ser	Gln	Ala	Ser	Ser	Gly	Gln	Ala	Arg	Met	Phe	Pro	Asn	Ala	Pro
			260					265					270	Tyr
Leu	Pro	Ser	Cys	Leu	Glu	Ser	Gln	Pro	Ala	Ile	Arg	Asn	Gln	Gly
		275						280					285	Tyr
Ser	Thr	Val	Thr	Phe	Asp	Gly	Thr	Pro	Ser	Tyr	Gly	His	Thr	Pro
	290					295					300			Ser
His	His	Ala	Ala	Gln	Phe	Pro	Asn	His	Ser	Phe	Lys	His	Glu	Asp
	305					310					315			320
Met	Gly	Gln	Gln	Gly	Ser	Leu	Gly	Glu	Gln	Gln	Tyr	Ser	Val	Pro
				325					330					335
Pro	Val	Tyr	Gly	Cys	His	Thr	Pro	Thr	Asp	Ser	Cys	Thr	Gly	Ser
			340					345					350	Gln
Ala	Leu	Leu	Leu	Arg	Thr	Pro	Tyr	Ser	Ser	Asp	Asn	Leu	Tyr	Gln
			355					360					365	Met
Thr	Ser	Gln	Leu	Glu	Cys	Met	Thr	Trp	Asn	Gln	Met	Asn	Leu	Gly
	370					375					380			Ala
Thr	Leu	Lys	Gly	His	Ser	Thr	Gly	Tyr	Glu	Ser	Asp	Asn	His	Thr
	385					390					395			400
Pro	Ile	Leu	Cys	Gly	Ala	Gln	Tyr	Arg	Ile	His	Thr	His	Gly	Val
				405					410					415
Arg	Gly	Ile	Gln											
			420											

&lt;210&gt; 394

&lt;211&gt; 362

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 394

Met	His	Ser	Phe	Ile	Lys	Gln	Glu	Pro	Ser	Trp	Gly	Gly	Ala	Glu	Pro
				5						10				15	

His Glu Glu Gln Cys Leu Ser Ala Phe Thr Val His Phe Ser Gly Gln

20										25					30				
Phe	Thr	Gly	Thr	Ala	Gly	Ala	Cys	Arg	Tyr	Gly	Pro	Phe	Gly	Pro	Pro				
		35					40					45							
Pro	Pro	Ser	Gln	Ala	Ser	Ser	Gly	Gln	Ala	Arg	Met	Phe	Pro	Asn	Ala				
	50					55					60								
Pro	Tyr	Leu	Pro	Ser	Cys	Leu	Glu	Ser	Gln	Pro	Ala	Ile	Arg	Asn	Gln				
	65				70					75					80				
Gly	Tyr	Ser	Thr	Val	Thr	Phe	Asp	Gly	Thr	Pro	Ser	Tyr	Gly	His	Thr				
				85					90					95					
Pro	Ser	His	His	Ala	Ala	Gln	Phe	Pro	Asn	His	Ser	Phe	Lys	His	Glu				
			100					105					110						
Asp	Pro	Met	Gly	Gln	Gln	Gly	Ser	Leu	Gly	Glu	Gln	Gln	Tyr	Ser	Val				
		115					120					125							
Pro	Pro	Pro	Val	Tyr	Gly	Cys	His	Thr	Pro	Thr	Asp	Ser	Cys	Thr	Gly				
	130					135					140								
Ser	Gln	Ala	Leu	Leu	Leu	Arg	Thr	Pro	Tyr	Ser	Ser	Asp	Asn	Leu	Tyr				
145					150					155					160				
Gln	Met	Thr	Ser	Gln	Leu	Glu	Cys	Met	Thr	Trp	Asn	Gln	Met	Asn	Leu				
				165					170					175					
Gly	Ala	Thr	Leu	Lys	Gly	His	Ser	Thr	Gly	Tyr	Glu	Ser	Asp	Asn	His				
			180					185					190						
Thr	Thr	Pro	Ile	Leu	Cys	Gly	Ala	Gln	Tyr	Arg	Ile	His	Thr	His	Gly				
		195					200						205						
Val	Phe	Arg	Gly	Ile	Gln	Asp	Val	Arg	Arg	Val	Pro	Gly	Val	Ala	Pro				
	210					215					220								
Thr	Leu	Val	Arg	Ser	Ala	Ser	Glu	Thr	Ser	Glu	Lys	Arg	Pro	Phe	Met				
225					230					235					240				
Cys	Ala	Tyr	Pro	Gly	Cys	Asn	Lys	Arg	Tyr	Phe	Lys	Leu	Ser	His	Leu				
				245					250					255					
Gln	Met	His	Ser	Arg	Lys	His	Thr	Gly	Glu	Lys	Pro	Tyr	Gln	Cys	Asp				
			260					265					270						
Phe	Lys	Asp	Cys	Glu	Arg	Arg	Phe	Phe	Arg	Ser	Asp	Gln	Leu	Lys	Arg				
		275					280					285							
His	Gln	Arg	Arg	His	Thr	Gly	Val	Lys	Pro	Phe	Gln	Cys	Lys	Thr	Cys				
	290					295					300								
Gln	Arg	Lys	Phe	Ser	Arg	Ser	Asp	His	Leu	Lys	Thr	His	Thr	Arg	Thr				
305					310					315					320				
His	Thr	Gly	Glu	Lys	Pro	Phe	Ser	Cys	Arg	Trp	Pro	Ser	Cys	Gln	Lys				
				325					330					335					

Lys Phe Ala Arg Ser Asp Glu Leu Val Arg His His Asn Met His Gln  
 340 345 350

Arg Asn Met Thr Lys Leu Gln Leu Ala Leu  
 355 360

<210> 395

<211> 214

<212> PRT

<213> Homo sapiens

<400> 395

Met His Ser Phe Ile Lys Gln Glu Pro Ser Trp Gly Gly Ala Glu Pro  
 5 10 15

His Glu Glu Gln Cys Leu Ser Ala Phe Thr Val His Phe Ser Gly Gln  
 20 25 30

Phe Thr Gly Thr Ala Gly Ala Cys Arg Tyr Gly Pro Phe Gly Pro Pro  
 35 40 45

Pro Pro Ser Gln Ala Ser Ser Gly Gln Ala Arg Met Phe Pro Asn Ala  
 50 55 60

Pro Tyr Leu Pro Ser Cys Leu Glu Ser Gln Pro Ala Ile Arg Asn Gln  
 65 70 75 80

Gly Tyr Ser Thr Val Thr Phe Asp Gly Thr Pro Ser Tyr Gly His Thr  
 85 90 95

Pro Ser His His Ala Ala Gln Phe Pro Asn His Ser Phe Lys His Glu  
 100 105 110

Asp Pro Met Gly Gln Gln Gly Ser Leu Gly Glu Gln Gln Tyr Ser Val  
 115 120 125

Pro Pro Pro Val Tyr Gly Cys His Thr Pro Thr Asp Ser Cys Thr Gly  
 130 135 140

Ser Gln Ala Leu Leu Leu Arg Thr Pro Tyr Ser Ser Asp Asn Leu Tyr  
 145 150 155 160

Gln Met Thr Ser Gln Leu Glu Cys Met Thr Trp Asn Gln Met Asn Leu  
 165 170 175

Gly Ala Thr Leu Lys Gly His Ser Thr Gly Tyr Glu Ser Asp Asn His  
 180 185 190

Thr Thr Pro Ile Leu Cys Gly Ala Gln Tyr Arg Ile His Thr His Gly  
 195 200 205

Val Phe Arg Gly Ile Gln  
 210

<210> 396  
<211> 30  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> PCR primer  
  
<400> 396  
gacgaaagca tatgcactcc ttcatcaaac 30  
  
<210> 397  
<211> 31  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> PCR primer  
  
<400> 397  
cgcggtgaatt catcactgaa tgcctctgaa g 31  
  
<210> 398  
<211> 31  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> PCR primer  
  
<400> 398  
cgataagcat atgacggccg cgtccgataa c 31  
  
<210> 399  
<211> 31  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> PCR primer  
  
<400> 399  
cgcggtgaatt catcactgaa tgcctctgaa g 31  
  
<210> 400  
<211> 31  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> PCR primer  
  
<400> 400  
cgataagcat atgacggccg cgtccgataa c 31  
  
<210> 401  
<211> 28  
<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer

<400> 401

gtctgcagcg gccgctcaaa gcgccagc

28

<210> 402

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer

<400> 402

gacgaaagca tatgcactcc ttcataaaac

30

<210> 403

<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer

<400> 403

gtctgcagcg gccgctcaaa gcgccagc

28

<210> 404

<211> 449

<212> PRT

<213> Homo sapiens

<400> 404

Met	Gly	Ser	Asp	Val	Arg	Asp	Leu	Asn	Ala	Leu	Leu	Pro	Ala	Val	Pro
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Ser	Leu	Gly	Gly	Gly	Gly	Gly	Cys	Ala	Leu	Pro	Val	Ser	Gly	Ala	Ala
		20					25						30		
Gln	Trp	Ala	Pro	Val	Leu	Asp	Phe	Ala	Pro	Pro	Gly	Ala	Ser	Ala	Tyr
	35					40					45				
Gly	Ser	Leu	Gly	Gly	Pro	Ala	Pro	Pro	Pro	Ala	Pro	Pro	Pro	Pro	Pro
	50					55				60					
Pro	Pro	Pro	Pro	His	Ser	Phe	Ile	Lys	Gln	Glu	Pro	Ser	Trp	Gly	Gly
	65				70				75					80	
Ala	Glu	Pro	His	Glu	Glu	Gln	Cys	Leu	Ser	Ala	Phe	Thr	Val	His	Phe
			85				90						95		
Ser	Gly	Gln	Phe	Thr	Gly	Thr	Ala	Gly	Ala	Cys	Arg	Tyr	Gly	Pro	Phe
		100					105						110		
Gly	Pro	Pro	Pro	Pro	Ser	Gln	Ala	Ser	Ser	Gly	Gln	Ala	Arg	Met	Phe
	115					120						125			
Pro	Asn	Ala	Pro	Tyr	Leu	Pro	Ser	Cys	Leu	Glu	Ser	Gln	Pro	Ala	Ile
	130					135					140				
Arg	Asn	Gln	Gly	Tyr	Ser	Thr	Val	Thr	Phe	Asp	Gly	Thr	Pro	Ser	Tyr
	145				150					155				160	
Gly	His	Thr	Pro	Ser	His	His	Ala	Ala	Gln	Phe	Pro	Asn	His	Ser	Phe

				165					170					175			
Lys	His	Glu	Asp	Pro	Met	Gly	Gln	Gln	Gly	Ser	Leu	Gly	Glu	Gln	Gln		
			180					185					190				
Tyr	Ser	Val	Pro	Pro	Pro	Val	Tyr	Gly	Cys	His	Thr	Pro	Thr	Asp	Ser		
		195					200					205					
Cys	Thr	Gly	Ser	Gln	Ala	Leu	Leu	Arg	Thr	Pro	Tyr	Ser	Ser	Asp			
	210					215				220							
Asn	Leu	Tyr	Gln	Met	Thr	Ser	Gln	Leu	Glu	Cys	Met	Thr	Trp	Asn	Gln		
225					230					235				240			
Met	Asn	Leu	Gly	Ala	Thr	Leu	Lys	Gly	Val	Ala	Ala	Gly	Ser	Ser	Ser		
				245					250					255			
Ser	Val	Lys	Trp	Thr	Glu	Gly	Gln	Ser	Asn	His	Ser	Thr	Gly	Tyr	Glu		
			260					265					270				
Ser	Asp	Asn	His	Thr	Thr	Pro	Ile	Leu	Cys	Gly	Ala	Gln	Tyr	Arg	Ile		
		275					280					285					
His	Thr	His	Gly	Val	Phe	Arg	Gly	Ile	Gln	Asp	Val	Arg	Arg	Val	Pro		
	290					295					300						
Gly	Val	Ala	Pro	Thr	Leu	Val	Arg	Ser	Ala	Ser	Glu	Thr	Ser	Glu	Lys		
305					310					315					320		
Arg	Pro	Phe	Met	Cys	Ala	Tyr	Pro	Gly	Cys	Asn	Lys	Arg	Tyr	Phe	Lys		
				325					330					335			
Leu	Ser	His	Leu	Gln	Met	His	Ser	Arg	Lys	His	Thr	Gly	Glu	Lys	Pro		
			340					345					350				
Tyr	Gln	Cys	Asp	Phe	Lys	Asp	Cys	Glu	Arg	Arg	Phe	Ser	Arg	Ser	Asp		
		355					360					365					
Gln	Leu	Lys	Arg	His	Gln	Arg	Arg	His	Thr	Gly	Val	Lys	Pro	Phe	Gln		
	370					375					380						
Cys	Lys	Thr	Cys	Gln	Arg	Lys	Phe	Ser	Arg	Ser	Asp	His	Leu	Lys	Thr		
385					390					395				400			
His	Thr	Arg	Thr	His	Thr	Gly	Lys	Thr	Ser	Glu	Lys	Pro	Phe	Ser	Cys		
				405					410					415			
Arg	Trp	Pro	Ser	Cys	Gln	Lys	Lys	Phe	Ala	Arg	Ser	Asp	Glu	Leu	Val		
			420					425					430				
Arg	His	His	Asn	Met	His	Gln	Arg	Asn	Met	Thr	Lys	Leu	Gln	Leu	Ala		
		435					440					445					

Leu

&lt;210&gt; 405

&lt;211&gt; 428

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 405

Met	Gly	Ser	Asp	Val	Arg	Asp	Leu	Asn	Ala	Leu	Leu	Pro	Ala	Val	Pro		
1				5					10					15			
Ser	Pro	Gly	Gly	Gly	Gly	Gly	Cys	Ala	Leu	Pro	Val	Ser	Gly	Ala	Thr		
		20					25						30				
Gln	Trp	Ala	Pro	Val	Leu	Asp	Phe	Val	Pro	Pro	Gly	Ala	Pro	Val	Cys		
		35					40					45					
Gly	Ser	Leu	Gly	Gly	Pro	Ala	Pro	Pro	Pro	Ala	Pro	Pro	Pro	Leu	Pro		
	50					55					60						
Pro	Pro	Pro	Ser	His	Ser	Phe	Thr	Lys	Gln	Glu	Pro	Ser	Trp	Gly	Gly		
65					70					75				80			
Thr	Glu	Pro	His	Ala	Gly	Gln	Gly	Arg	Ser	Ala	Leu	Val	Ala	His	Ser		
				85					90					95			
Ser	Gly	Gln	Phe	Thr	Gly	Thr	Ala	Gly	Ala	Cys	Arg	Tyr	Gly	Pro	Phe		

			100					105					110		
Gly	Pro	Pro	Pro	Pro	Ser	Gln	Ala	Ser	Ser	Gly	Gln	Ala	Arg	Met	Phe
		115					120					125			
Pro	Asn	Ala	Pro	Tyr	Leu	Pro	Ser	Cys	Leu	Glu	Ser	Gln	Pro	Ala	Ile
	130					135					140				
Arg	Asn	Gln	Gly	Tyr	Ser	Thr	Val	Thr	Phe	Asp	Gly	Thr	Pro	Ser	Tyr
145					150					155					160
Gly	His	Thr	Pro	Ser	His	His	Ala	Ala	Gln	Phe	Pro	Asn	His	Ser	Ser
				165					170					175	
Lys	His	Glu	Asp	Pro	Met	Gly	Gln	Gln	Gly	Ser	Pro	Gly	Glu	Gln	Gln
			180					185					190		
Tyr	Ser	Ala	Pro	Pro	Pro	Val	Cys	Gly	Cys	Arg	Thr	Pro	Thr	Gly	Ser
		195					200					205			
Cys	Thr	Gly	Ser	Gln	Ala	Leu	Leu	Leu	Arg	Ala	Pro	Tyr	Ser	Gly	Gly
	210					215					220				
Asp	Leu	His	Gln	Thr	Thr	Ser	Gln	Leu	Gly	His	Met	Ala	Trp	Asn	Gln
225					230					235					240
Thr	Asn	Leu	Gly	Ala	Thr	Leu	Lys	Gly	His	Gly	Thr	Gly	Tyr	Glu	Ser
				245					250					255	
Asp	Asp	His	Thr	Thr	Pro	Ile	Leu	Cys	Gly	Thr	Gln	Tyr	Arg	Ile	Arg
			260					265					270		
Ala	Arg	Gly	Val	Leu	Arg	Gly	Thr	Gln	Asp	Val	Arg	Cys	Val	Pro	Gly
		275					280					285			
Val	Ala	Pro	Thr	Leu	Val	Arg	Ser	Ala	Ser	Glu	Thr	Ser	Glu	Lys	Arg
	290					295					300				
Pro	Leu	Met	Cys	Ala	Tyr	Pro	Gly	Cys	Asn	Lys	Arg	His	Phe	Lys	Pro
305					310					315					320
Ser	Arg	Leu	Arg	Val	Arg	Gly	Arg	Glu	Arg	Thr	Gly	Glu	Lys	Pro	Tyr
				325					330					335	
Gln	Arg	Asp	Phe	Lys	Asp	Arg	Gly	Arg	Gly	Leu	Leu	Arg	Pro	Asp	Gln
			340						345				350		
Leu	Lys	Arg	His	Gln	Arg	Gly	His	Thr	Gly	Val	Lys	Pro	Leu	Gln	Cys
		355					360					365			
Glu	Ala	Arg	Arg	Arg	Pro	Pro	Arg	Pro	Gly	His	Leu	Lys	Val	His	Thr
		370				375				380					
Arg	Thr	His	Thr	Gly	Gly	Glu	Pro	Phe	Ser	Cys	Arg	Trp	Pro	Ser	Cys
385					390					395					400
Gln	Glu	Lys	Ser	Ala	Arg	Pro	Asp	Glu	Ser	Ala	Arg	Arg	His	Asn	Met
				405					410					415	
His	Gln	Arg	Asn	Met	Thr	Lys	Leu	Gln	Leu	Ala	Leu				
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<210> 406
<211> 414
<212> PRT
<213> Homo sapiens
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      20          25          30

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Gln Trp Ala Pro Val Leu Asp Phe Ala Pro Pro Gly Ala Ser Ala His  
 35 40 45  
 Gly Pro Leu Gly Gly Pro Ala Pro Pro Ser Ala Pro Pro Pro Pro  
 50 55 60  
 Pro Pro Pro Pro His Ser Phe Ile Lys Gln Gly Pro Ser Trp Gly Gly  
 65 70 75 80  
 Ala Glu Leu His Xaa Xaa Gln Tyr Leu Ser Ala Phe Thr Val His Ser  
 85 90 95  
 Ser Gly Gln Val His Trp His Gly Arg Gly Leu Ser Leu Arg Ala Pro  
 100 105 110  
 Arg Pro Pro Ser Ala Gln Pro Gly Val Ile Arg Pro Gly Gln Asp Val  
 115 120 125  
 Ser Arg Ala Leu Pro Ala Gln Pro Pro Arg Glu Pro Ala Arg Tyr Pro  
 130 135 140  
 Gln Ser Gly Leu Gln His Gly His Leu Arg Arg Gly Val Arg Leu Arg  
 145 150 155 160  
 Ser His Ala Leu Ala Pro Cys Gly Ala Val Leu Xaa Xaa Thr Arg Ala  
 165 170 175  
 Gly Ser His Gly Pro Ala Gly Ser Ala Gly Ala Ala Val Leu Gly Ala  
 180 185 190  
 Ala Pro Gly Leu Trp Pro Pro His Pro Arg Arg Gln Leu Arg Arg Gln  
 195 200 205  
 Pro Gly Phe Ala Ala Glu Gly Ala Leu Gln Arg Arg Phe Ile Pro Ser  
 210 215 220  
 Asp Val Pro Ala Val His Gly Leu Glu Ser Asp Glu Pro Arg Gly Arg  
 225 230 235 240  
 Leu Xaa Gly Pro Xaa Xaa Xaa Val Arg Glu Arg Ser His Asn Ala Arg  
 245 250 255  
 Pro Leu Arg Ser Pro Ile Gln Asn Thr His Ala Arg Cys Leu Gln Gly  
 260 265 270  
 Arg Ser Gly Cys Ala Pro Cys Ala Trp Ser Ser Pro Asp Ser Cys Thr  
 275 280 285  
 Val Gly Ile Gly Gln Gly Thr Pro Pro His Val Cys Leu Pro Arg Leu  
 290 295 300  
 Gln Glu Val Ser Glu Ala Ala Pro Leu Thr Asp Ala Arg Glu Ala Arg  
 305 310 315 320  
 Trp Glu Thr Ile Pro Val Leu Gln Gly Leu Trp Thr Glu Val Phe Leu  
 325 330 335  
 Leu Arg Pro Ala Gln Lys Thr Pro Gly Glu Ala Tyr Arg Cys Glu Ala  
 340 345 350  
 Ile Pro Ala Asp Leu Ser Ala Arg Val Leu Pro Ala Gln Pro Pro Glu  
 355 360 365  
 Asp Pro Arg Gln Asp Ser Cys Arg Lys Ala Pro Gln Leu Ser Val Val  
 370 375 380  
 Arg Leu Ser Glu Lys Ala Cys Pro Val Lys Val Gly Pro Pro Ser Arg  
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 His Ala Ser Glu Gly His Asp Arg Thr Pro Ala Gly Ala Leu  
 405 410

&lt;210&gt; 407

&lt;211&gt; 417

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 407

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 1 5 10 15

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 20 25 30  
 Gln Trp Ala Pro Val Pro Ala Ser Ala Pro Pro Gly Ala Ser Ala Tyr  
 35 40 45  
 Asp Ser Leu Gly Gly Pro Ala Pro Pro Pro Ala Pro Pro Pro Pro  
 50 55 60  
 Pro Pro Pro Pro His Ser Cys Gly Glu Gln Gly Pro Ser Trp Gly Gly  
 65 70 75 80  
 Ala Glu Pro Arg Glu Gly Gln Cys Leu Ser Ala Pro Ala Val Arg Phe  
 85 90 95  
 Ser Gly Arg Phe Thr Gly Thr Val Gly Ala Cys Arg Tyr Gly Pro Leu  
 100 105 110  
 Gly Pro Pro Pro Ser Gln Ala Pro Ser Gly Gln Thr Arg Met Leu  
 115 120 125  
 Pro Ser Ala Pro Tyr Leu Ser Ser Cys Leu Arg Ser Arg Ser Ala Ile  
 130 135 140  
 Arg Ser Gln Gly Arg Ser Thr Ala Pro Ser Ala Gly Arg Pro Ala Met  
 145 150 155 160  
 Ala Pro Thr Leu Ala Pro Pro Ala Gln Ser His Tyr Ser Gln His Gly  
 165 170 175  
 Val Leu His Gly Pro Ala Gly Leu Ala Gly Ala Ala Val Leu Gly Ala  
 180 185 190  
 Ala Pro Gly Leu Trp Leu Pro His Pro His Arg Gln Leu His Arg Gln  
 195 200 205  
 Pro Gly Phe Ala Ala Glu Asp Ala Leu Gln Gln Gln Phe Ile Pro Asn  
 210 215 220  
 Asp Ile Pro Ala Met His Asp Leu Glu Ser Asp Glu Leu Arg Ser His  
 225 230 235 240  
 Leu Lys Gly Pro Gln His Arg Val Arg Glu Arg Pro His Asn Ala His  
 245 250 255  
 Pro Leu Arg Ser Pro Ile Gln Asn Thr His Ala Arg Cys Leu Gln Arg  
 260 265 270  
 His Ser Gly Cys Ala Thr Cys Ala Trp Ser Ser Pro Asp Ser Cys Thr  
 275 280 285  
 Val Ala Pro Glu Thr Ser Glu Asn Ala Pro Trp Cys Val Leu Pro Gly  
 290 295 300  
 Leu Gln Gly Val Phe Ala Val Pro Leu Thr Gly Ala Gln Gln Glu Ala  
 305 310 315 320  
 His Trp Asp Ala Thr Pro Val Arg Leu Gln Gly Pro Trp Thr Arg Ala  
 325 330 335  
 Ser Pro Phe Gly Thr Ser Pro Arg Asp Thr Lys Gly Asp Ile Gln Val  
 340 345 350  
 Arg Asn His Ser Ser Val Arg Leu Val Ser Glu Gly Ser Pro Gly Pro  
 355 360 365  
 Thr Thr Gly Pro Thr Pro Gly Pro Thr Arg Val Gly Ser Pro Ser Ala  
 370 375 380  
 Ala Gly Gly Gln Ala Ala Arg Glu Gly Ser Pro Ser Gln Thr Asn Ser  
 385 390 395 400  
 Val Ile Thr Thr Cys Ile Ser Glu Thr Leu Asn Ser Ser Trp Arg Phe  
 405 410 415  
 Glu

&lt;210&gt; 408

&lt;211&gt; 429

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 408

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Gln Trp Ala Pro Val Leu Asp Phe Ala Pro Pro Gly Ala Ser Ala Tyr
35      40      45
Gly Ser Leu Gly Gly Pro Ala Pro Pro Pro Ala Pro Pro Pro Pro
50      55      60
Pro Pro Pro Pro His Ser Phe Ile Lys Gln Glu Pro Ser Trp Gly Gly
65      70      75      80
Ala Glu Pro His Glu Glu Gln Cys Leu Ser Ala Phe Thr Val His Phe
85      90      95
Ser Gly Gln Phe Thr Gly Thr Ala Gly Ala Cys Arg Tyr Gly Pro Phe
100     105     110
Gly Pro Pro Pro Pro Ser Gln Ala Ser Ser Gly Gln Ala Arg Met Phe
115     120     125
Pro Asn Ala Pro Tyr Leu Pro Ser Cys Leu Glu Ser Gln Pro Ala Ile
130     135     140
Arg Asn Gln Gly Tyr Ser Thr Val Thr Phe Asp Gly Thr Pro Ser Tyr
145     150     155     160
Gly His Thr Pro Ser His His Ala Ala Gln Phe Pro Asn His Ser Phe
165     170     175
Lys His Glu Asp Pro Met Gly Gln Gln Gly Ser Leu Gly Glu Gln Gln
180     185     190
Tyr Ser Val Pro Pro Pro Val Tyr Gly Cys His Thr Pro Thr Asp Ser
195     200     205
Cys Thr Gly Ser Gln Ala Leu Leu Leu Arg Thr Pro Tyr Ser Ser Asp
210     215     220
Asn Leu Tyr Gln Met Thr Ser Gln Leu Glu Cys Met Thr Trp Asn Gln
225     230     235     240
Met Asn Leu Gly Ala Thr Leu Lys Gly His Ser Thr Gly Tyr Glu Ser
245     250     255
Asp Asn His Thr Thr Pro Ile Leu Cys Gly Ala Gln Tyr Arg Ile His
260     265     270
Thr His Gly Val Phe Arg Gly Ile Gln Asp Val Arg Arg Val Pro Gly
275     280     285
Val Ala Pro Thr Leu Val Arg Ser Ala Ser Glu Thr Ser Glu Lys Arg
290     295     300
Pro Phe Met Cys Ala Tyr Pro Gly Cys Asn Lys Arg Tyr Phe Lys Leu
305     310     315     320
Ser His Leu Gln Met His Ser Arg Lys His Thr Gly Glu Lys Pro Tyr
325     330     335
Gln Cys Asp Phe Lys Asp Cys Glu Arg Arg Phe Phe Arg Ser Asp Gln
340     345     350
Leu Lys Arg His Gln Arg Arg His Thr Gly Val Lys Pro Phe Gln Cys
355     360     365
Lys Thr Cys Gln Arg Lys Phe Ser Arg Ser Asp His Leu Lys Thr His
370     375     380
Thr Arg Thr His Thr Gly Glu Lys Pro Phe Ser Cys Arg Trp Pro Ser
385     390     395     400
Cys Gln Lys Lys Phe Ala Arg Ser Asp Glu Leu Val Arg His His Asn
405     410     415
Met His Gln Arg Asn Met Thr Lys Leu Gln Leu Ala Leu
420     425

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<210> 409  
 <211> 495  
 <212> PRT  
 <213> Homo sapiens

<400> 409

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			20					25					30		
Asp	Val	Arg	Asp	Leu	Asn	Ala	Leu	Leu	Pro	Ala	Val	Pro	Ser	Leu	Gly
		35					40					45			
Gly	Gly	Gly	Gly	Cys	Ala	Leu	Pro	Val	Ser	Gly	Ala	Ala	Gln	Trp	Ala
	50					55					60				
Pro	Val	Leu	Asp	Phe	Ala	Pro	Pro	Gly	Ala	Ser	Ala	Tyr	Gly	Ser	Leu
65					70					75					80
Gly	Gly	Pro	Ala	Pro	Pro	Pro	Ala	Pro	Pro	Pro	Pro	Pro	Pro	Pro	His
				85					90					95	
Ser	Phe	Ile	Lys	Gln	Glu	Pro	Ser	Trp	Gly	Gly	Ala	Glu	Pro	His	Glu
			100					105					110		
Glu	Gln	Cys	Leu	Ser	Ala	Phe	Thr	Val	His	Phe	Ser	Gly	Gln	Phe	Thr
		115					120					125			
Gly	Thr	Ala	Gly	Ala	Cys	Arg	Tyr	Gly	Pro	Phe	Gly	Pro	Pro	Pro	Pro
	130					135					140				
Ser	Gln	Ala	Ser	Ser	Gly	Gln	Ala	Arg	Met	Phe	Pro	Asn	Ala	Pro	Tyr
145					150					155					160
Leu	Pro	Ser	Cys	Leu	Glu	Ser	Gln	Pro	Ala	Ile	Arg	Asn	Gln	Gly	Tyr
			165						170					175	
Ser	Thr	Val	Thr	Phe	Asp	Gly	Thr	Pro	Ser	Tyr	Gly	His	Thr	Pro	Ser
			180					185					190		
His	His	Ala	Ala	Gln	Phe	Pro	Asn	His	Ser	Phe	Lys	His	Glu	Asp	Pro
		195					200					205			
Met	Gly	Gln	Gln	Gly	Ser	Leu	Gly	Glu	Gln	Gln	Tyr	Ser	Val	Pro	Pro
	210					215					220				
Pro	Val	Tyr	Gly	Cys	His	Thr	Pro	Thr	Asp	Ser	Cys	Thr	Gly	Ser	Gln
225					230					235					240
Ala	Leu	Leu	Leu	Arg	Thr	Pro	Tyr	Ser	Ser	Asp	Asn	Leu	Tyr	Gln	Met
				245					250					255	
Thr	Ser	Gln	Leu	Glu	Cys	Met	Thr	Trp	Asn	Gln	Met	Asn	Leu	Gly	Ala
			260					265					270		
Thr	Leu	Lys	Gly	His	Ser	Thr	Gly	Tyr	Glu	Ser	Asp	Asn	His	Thr	Thr
		275					280					285			
Pro	Ile	Leu	Cys	Gly	Ala	Gln	Tyr	Arg	Ile	His	Thr	His	Gly	Val	Phe
	290					295					300				
Arg	Gly	Ile	Gln	Asp	Val	Arg	Arg	Val	Pro	Gly	Val	Ala	Pro	Thr	Leu
305					310					315					320
Val	Arg	Ser	Ala	Ser	Glu	Thr	Ser	Glu	Lys	Arg	Pro	Phe	Met	Cys	Ala
				325					330					335	
Tyr	Pro	Gly	Cys	Asn	Lys	Arg	Tyr	Phe	Lys	Leu	Ser	His	Leu	Gln	Met
			340					345					350		
His	Ser	Arg	Lys	His	Thr	Gly	Glu	Lys	Pro	Tyr	Gln	Cys	Asp	Phe	Lys
		355					360					365			
Asp	Cys	Glu	Arg	Arg	Phe	Phe	Arg	Ser	Asp	Gln	Leu	Lys	Arg	His	Gln
	370					375					380				
Arg	Arg	His	Thr	Gly	Val	Lys	Pro	Phe	Gln	Cys	Lys	Thr	Cys	Gln	Arg
385					390					395					400
Lys	Phe	Ser	Arg	Ser	Asp	His	Leu	Lys	Thr	His	Thr	Arg	Thr	His	Thr
				405					410					415	

Gly	Glu	Lys	Pro	Phe	Ser	Cys	Arg	Trp	Pro	Ser	Cys	Gln	Lys	Lys	Phe
			420					425					430		
Ala	Arg	Ser	Asp	Glu	Leu	Val	Arg	His	His	Asn	Met	His	Gln	Arg	Asn
		435					440					445			
Met	Thr	Lys	Leu	Gln	Leu	Ala	Leu	Leu	Asn	Asn	Met	Leu	Ile	Pro	Ile
	450					455					460				
Ala	Val	Gly	Gly	Ala	Leu	Ala	Gly	Leu	Val	Leu	Ile	Val	Leu	Ile	Ala
465					470					475					480
Tyr	Leu	Ile	Gly	Arg	Lys	Arg	Ser	His	Ala	Gly	Tyr	Gln	Thr	Ile	
				485					490					495	

&lt;210&gt; 410

&lt;211&gt; 504

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 410

Met	Gln	Ile	Phe	Val	Lys	Thr	Leu	Thr	Gly	Lys	Thr	Ile	Thr	Leu	Glu
1				5					10					15	
Val	Glu	Pro	Ser	Asp	Thr	Ile	Glu	Asn	Val	Lys	Ala	Lys	Ile	Gln	Asp
			20					25					30		
Lys	Glu	Gly	Ile	Pro	Pro	Asp	Gln	Gln	Arg	Leu	Ile	Phe	Ala	Gly	Lys
	35						40					45			
Gln	Leu	Glu	Asp	Gly	Arg	Thr	Leu	Ser	Asp	Tyr	Asn	Ile	Gln	Lys	Glu
	50					55					60				
Ser	Thr	Leu	His	Leu	Val	Leu	Arg	Leu	Arg	Gly	Ala	Met	Gly	Ser	Asp
65				70						75				80	
Val	Arg	Asp	Leu	Asn	Ala	Leu	Leu	Pro	Ala	Val	Pro	Ser	Leu	Gly	Gly
			85						90					95	
Gly	Gly	Gly	Cys	Ala	Leu	Pro	Val	Ser	Gly	Ala	Ala	Gln	Trp	Ala	Pro
			100					105					110		
Val	Leu	Asp	Phe	Ala	Pro	Pro	Gly	Ala	Ser	Ala	Tyr	Gly	Ser	Leu	Gly
	115						120					125			
Gly	Pro	Ala	Pro	Pro	Pro	Ala	Pro	Pro	Pro	Pro	Pro	Pro	Pro	Pro	His
	130					135						140			
Ser	Phe	Ile	Lys	Gln	Glu	Pro	Ser	Trp	Gly	Gly	Ala	Glu	Pro	His	Glu
145					150					155					160
Glu	Gln	Cys	Leu	Ser	Ala	Phe	Thr	Val	His	Phe	Ser	Gly	Gln	Phe	Thr
			165						170					175	
Gly	Thr	Ala	Gly	Ala	Cys	Arg	Tyr	Gly	Pro	Phe	Gly	Pro	Pro	Pro	Pro
			180					185					190		
Ser	Gln	Ala	Ser	Ser	Gly	Gln	Ala	Arg	Met	Phe	Pro	Asn	Ala	Pro	Tyr
	195						200					205			
Leu	Pro	Ser	Cys	Leu	Glu	Ser	Gln	Pro	Ala	Ile	Arg	Asn	Gln	Gly	Tyr
	210					215						220			
Ser	Thr	Val	Thr	Phe	Asp	Gly	Thr	Pro	Ser	Tyr	Gly	His	Thr	Pro	Ser
225					230					235					240
His	His	Ala	Ala	Gln	Phe	Pro	Asn	His	Ser	Phe	Lys	His	Glu	Asp	Pro
				245					250					255	
Met	Gly	Gln	Gln	Gly	Ser	Leu	Gly	Glu	Gln	Gln	Tyr	Ser	Val	Pro	Pro
			260					265					270		
Pro	Val	Tyr	Gly	Cys	His	Thr	Pro	Thr	Asp	Ser	Cys	Thr	Gly	Ser	Gln
			275				280					285			
Ala	Leu	Leu	Leu	Arg	Thr	Pro	Tyr	Ser	Ser	Asp	Asn	Leu	Tyr	Gln	Met
	290					295					300				
Thr	Ser	Gln	Leu	Glu	Cys	Met	Thr	Trp	Asn	Gln	Met	Asn	Leu	Gly	Ala
305					310					315					320

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Thr Leu Lys Gly His Ser Thr Gly Tyr Glu Ser Asp Asn His Thr Thr
      325      330      335
Pro Ile Leu Cys Gly Ala Gln Tyr Arg Ile His Thr His Gly Val Phe
      340      345      350
Arg Gly Ile Gln Asp Val Arg Arg Val Pro Gly Val Ala Pro Thr Leu
      355      360      365
Val Arg Ser Ala Ser Glu Thr Ser Glu Lys Arg Pro Phe Met Cys Ala
      370      375      380
Tyr Pro Gly Cys Asn Lys Arg Tyr Phe Lys Leu Ser His Leu Gln Met
      385      390      395      400
His Ser Arg Lys His Thr Gly Glu Lys Pro Tyr Gln Cys Asp Phe Lys
      405      410      415
Asp Cys Glu Arg Arg Phe Phe Arg Ser Asp Gln Leu Lys Arg His Gln
      420      425      430
Arg Arg His Thr Gly Val Lys Pro Phe Gln Cys Lys Thr Cys Gln Arg
      435      440      445
Lys Phe Ser Arg Ser Asp His Leu Lys Thr His Thr Arg Thr His Thr
      450      455      460
Gly Glu Lys Pro Phe Ser Cys Arg Trp Pro Ser Cys Gln Lys Lys Phe
      465      470      475      480
Ala Arg Ser Asp Glu Leu Val Arg His His Asn Met His Gln Arg Asn
      485      490      495
Met Thr Lys Leu Gln Leu Ala Leu
      500

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<210> 411
<211> 10
<212> PRT
<213> Homo sapiens

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<400> 411
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<210> 412
<211> 15
<212> PRT
<213> Homo sapiens

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<400> 412
Gln Trp Ala Pro Val Leu Asp Phe Ala Pro Pro Gly Ala Ser Ala
  1              5              10              15

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<210> 413
<211> 15
<212> PRT
<213> Homo sapiens

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<400> 413
Val Leu Asp Phe Ala Pro Pro Gly Ala Ser Ala Tyr Gly Ser Leu
  1              5              10              15

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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/31139

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) :Please See Extra Sheet.

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350, 300, 828, 387.7, 388.85, 387.9; 536/23.5, 24.3; 514/2; 435/6, 7.1, 7.7, 320.1, 372.3; 424/277.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST 2.0, MEDICINE BIOTECH databases from DIALOG search terms: inventor names, wt1, peptid?, fusion, nucleic antibod?

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 00/18795 A2 (CORIXA CORPORATION) 6 APRIL 2000, see entire document.	1-34

☐ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:		"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 13 DECEMBER 2001	Date of mailing of the international search report 13 FEB 2002
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer <i>Felicia D. Roberts for</i> RON SCHWADRON Telephone No. (703) 308-0196

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/51139

## A. CLASSIFICATION OF SUBJECT MATTER:

IPC (7):

A61K 38/16, 38/17; C07H 21/02, 21/04; C07K 14/435; C12N 5/10; G01N 33/53

## A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

530/350, 300, 828, 387.7, 388.85, 387.9; 536/23.5, 24.3; 514/2; 495/6, 7.1, 7.7, 320.1, 372.3; 424/277.1